Alternative Testing Systems for Evaluating Noncarcinogenic, Hematologic Toxicity

Ralph E. Parchment

Division of Hematology and Oncology, the Karmanos Cancer Institute, Wayne State University, Detroit, Michigan

Hematopoietic tissues are the targets of numerous xenobiotics. Clinical hematotoxicity is either a decrease or an increase in peripheral blood cell counts in one or more cell lineages—a cytopenia or a cytosis, respectively—that carries a risk of an adverse clinical event. The purpose of *in vitro* hematotoxicology is the prediction of these adverse hematologic effects from the effects of the toxicants on human hematopoietic targets under controlled experimental conditions in the laboratory. Building on its important foundations in experimental hematology and the wealth of hematotoxicology data found in experimental oncology, this field of alternative toxicology has developed rapidly during the past decade. Although the colony-forming unit–granulocyte/monocyte neutrophil progenitor is most frequently evaluated, other defined progenitors and stem cells as well as cell types found in the marrow stroma can be evaluated *in vitro*. End points have been proposed for predicting toxicant exposure levels at the maximum tolerated dose and the no observable adverse effect level for the neutrophil lineage, and several clinical prediction models for neutropenia have developed to the point that they are ready for prospective evaluation and validation in both preclinical species and humans. Known predictive end points are the key to successful comparisons across species or across chemical structures when *in vitro* dose–response curves are nonparallel. Analytical chemistry support is critical for accurate interpretation of *in vitro* data and for relating the *in vitro* pharmacodynamics to the *in vivo* pharmacokinetics. In contrast to acute neutropenia, anemia and acute thrombocytopenia, as well as adverse effects from chronic toxicant exposure, are much more difficult to predict from *in vitro* data. Pharmacologic principles critical for clinical predictions from *in vitro* data very likely will apply to toxicities to other proliferative tissues, such as mucositis. — *Environ Health Perspect* 106(Suppl 2):541–557 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-2/541-557parchment/abstract.html*

Key words: neutropenia, thrombocytopenia, myelosuppression, myelotoxicity, hematotoxicity, hematotoxicology, *in vitro* assay, stem cells, hematopoiesis, progenitor cells, CFU-GM, NOAEL, permissible exposure limit, MTD, toxicology, IND, NDA

In Vitro Hematotoxicology as an Alternative Toxicology

Clinical toxicology can be defined as the study of the clinically significant perturbations, caused by xenobiotic and/or therapeutic exposure, which are adverse in nature (harmful) for the patient. This paper focuses on the prediction of clinically significant, adverse perturbations in peripheral blood cell counts. It addresses benign lesions in the hematopoietic system but does not consider leukemogenesis.

The field of hematotoxicology includes the study of adverse effects of toxicants on mature blood cells and also the precursor cells in the hematopoietic (blood forming) tissues. There are established techniques for assessing adverse effects of xenobiotics on mature blood cells. More recently, the availability of recombinant hematopoietic growth factors makes possible the evaluation of adverse effects against the blood-forming precursor cells as well. Thus, it is now possible to study human hematotoxicology in the preclinical setting. Because it is possible to study the effects of a toxicant on its actual target cell, it seems reasonable to expect *in vitro* hematotoxicology to be highly predictive.

Fundamentally, toxicology has two goals: identification of the tissues that are susceptible to the toxic effects of the xenobiotic and determination of the level of acute and chronic exposures (doses) that these tissues can tolerate without clinical consequences. The first goal is qualitative; it necessarily involves comparative toxicology in multiple tissues. This comparison is most efficiently completed *in vivo* where all organs can be exposed simultaneously. A similar evaluation performed *ex vivo* necessarily requires the assay of multiple human tissues under identical conditions, which at the present time is not technically possible. The estimation of the acceptable level of human exposure from *in vitro* hematotoxicology data assumes that the toxicant's most potent effects are toward the bone marrow, i.e., hematopoietic tissue is the most sensitive of the human tissues to toxicity. By its nature, hematotoxicology complements, refines, and actually improves standard toxicology testing (usually *in vivo*), which still is required to identify hematopoiesis as the most likely tissue target of the toxicant in humans. The second goal is quantitative and estimates the level of toxicant exposure that can be tolerated by the target tissue. This is critical for accurate risk assessment and establishment of reasonable regulatory limits on exposure. In this case, *ex vivo* evaluation must involve evaluation of toxicity to only the target tissue or cells derived from it and *in vitro* hematotoxicology meets this second goal.

The laboratory techniques for evaluating the effects of a toxicant on human hematopoietic tissue are relatively straightforward, and there is a wealth of data on the toxic effects of xenobiotics on hematopoietic target cells. Most laboratories have the

This paper was prepared as background for the 13th Meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC): Alternative Testing Methodologies held 26–31 January 1997 in Ispra, Italy. Manuscript received at *EHP* 9 May 1997; accepted 5 November 1997.

Many of the ideas in this paper germinated during very stimulating discussions with C.K. Grieshaber and J.M. Collins at the U.S. FDA and J.E. Tomaszewski at the U.S. NCI. I also thank two key Phase I investigators, L.M. Ghielmini (Lugano) and P.M. LoRusso (Detroit), for their openness in considering this field as an integral part of Phase I clinical development. Finally, gratitude is due for the expert assistance of technicians in Dayton, OH, Washington, D.C., Detroit, MI, and elsewhere who have made these data possible. This work supported in part by U.S. National Institutes of Health grants CA62487, CA46560, CA75283, and a contract Consorzio Milano Richerche.

Address correspondence to Dr. R.E. Parchment, Division of Hematology and Oncology, Wayne State University, Harper Hospital, 3990 John R. Street, Detroit, MI. Telephone: (313) 745-9519. Fax: (313) 993-0559. E-mail: parchmen@med.wayne.edu

Abbreviations used: AUC, area under the plasma concentration versus time curve; AZT, azidothymidine; BFU, burst-forming unit-erythroid; CFU, colony-forming unit; CFU-f, CFU-fibroblastoid; CFU-GEMM, CFU-granulocyte, erythroid, megakaryocyte, and monocyte; CFU-GM, CFU-granulocyte/monocyte; CFU-Mk, CFU-megakaryocyte; CYP450, cytochrome P450; ECVAM, European Centre for the Validation of Alternative Methods; HPP-CFC, high proliferating protential–colony-forming cell; IC, inhibition concentration (number in subscript indicates percent of inhibition produced); IL, interleukin; LTC-IC, long-term culture-initiating cell; MTC, maximum tolerated concentration; MTD, maximum tolerated dose; NOAEC, no observable adverse effect concentration; NOAEL, no observable adverse effect level; PEL, permissible exposure limit; TGF-β, transforming growth factor beta.

capability of performing all of the *ex vivo* tests described here. In contrast, the interpretation of the data and the quantitative prediction of the acceptable level of clinical exposure from the data are much more difficult. It is this latter arena in which we have specialized. We have investigated prediction models that translate the data into predicted hematologic perturbations as a function of toxicant exposure levels. The goal in the regulatory setting usually emphasizes the prediction of two levels of exposure: the highest dose that will not cause a clinically adverse effect and the dose that causes maximally tolerated, reversible perturbations in peripheral blood counts. The former is often termed the no observable adverse effect level (NOAEL), whereas the latter is termed the maximum tolerated dose (MTD). Human exposure at the MTD is undesirable except for anticancer agents; with this exception, permissible exposure limits (PELs) are set for all regulated products from the NOAEL.

Substantial pharmacologic issues arise during the prediction of clinical outcomes from *in vitro* data. *Ex vivo* assays determine the concentration and exposure duration that cause toxicity. In contrast, the regulation of human exposure requires that decisions be based on units of dose or dose intensity (i.e., mg/m^2 or mg/kg per unit of time). The prediction of a dose that produces the *in vitro* concentration–time–effect relationship *in vivo* involves pharmacokinetics—the disposition of xenobiotic throughout the body. By analogy to animal and clinical toxicology, *in vitro* hematotoxicology aims to determine the no observable adverse effect concentration (NOAEC) and the maximum tolerated concentration (MTC) of toxicant under a specific schedule of exposure. The pharmacokineticist can then calculate the NOAEL and MTD doses that produce these NOAECs and MTCs. The NOAEC and the MTC are two end points useful in regulatory science sought during *in vitro* hematotoxicology studies.

The success to date lies primarily in the identification of the *in vitro* inhibition concentration value that is the MTC. An international validation study sponsored by the European Centre for the Validation of Alternative Methods (ECVAM) is underway to evaluate the predictive value of this putative MTC for clinical neutropenia (*1*). Results from this formal evaluation of *in vitro* hematotoxicology can be expected by January 1999. Because a wealth of detailed preclinical and clinical pharmacokinetic and hematotoxicity data exists for antineoplastic agents, these compounds serve as prototype hematotoxicants to elucidate the principles of predicting the NOAEC and the MTC. Once the prediction models are developed with antineoplastics, it is expected that they will generally be applicable to all other xenobiotics.

The Hematopoietic System as a Prototype for *in Vivo* and *in Vitro* Toxicology

Circulating Blood Cells and the *in Vivo* End Point

The blood of mammals contains a variety of differentiated cell types with specific functions. Red blood cells (erythrocytes) deliver oxygen; platelets contribute to clot formation; and lymphocytes, monocytes, and granulocytes provide resistance to infectious organisms and foreign materials. The granulocytes and platelets have a half-life in the circulation of only a few hours and a few days, respectively, whereas erythrocytes have a much longer half-life of several months. The proportion of white blood cells that are lymphocytes or granulocytes differs across mammalian species, and this must be kept in mind when interpreting toxicologic data. For example, rodents exhibit a lymphocyte:granulocyte ratio of about 4:1 whereas humans and dogs show a 1:4 ratio. A toxicant that causes severe neutropenia in the absence of lymphocytopenia will only be detected during rodent toxicology studies if differential counts are made on the leukocyte population. Otherwise, the maximum adverse effect will be a 20% reduction in white blood cell counts, which is likely to be considered unremarkable. Furthermore, it is important that validation studies of laboratory end points correlate the *in vitro* data to the correct *in vivo* end point. Assays to predict neutropenia need to be correlated with neutrophil counts, not with leukocyte counts.

Hematopoietic Cells as Producers of New Blood Cells

New blood cells must constantly be produced to maintain the peripheral blood cell counts, and under healthy physiologic conditions there is a balance between new cell production and cell loss that leads to the constancy of blood cell counts observed clinically. The hematopoietic tissues that produce new blood cells are primarily located in the bone marrow in humans but in both bone marrow and spleen in rodents and sometimes dogs. Hematopoietic tissue contains a continuum of increasingly differentiated elements within all blood cell lineages. Developing myeloid, erythroid, megakaryocytic, and lymphoid cells can be distinguished cytologically by the trained eye or with histochemistry for lineage-restricted features. The highly undifferentiated cells are progenitors of the immature blood cells. There are also hematopoietic stem cells in this undifferentiated population. Fixed stromal cells (both fibroblastic and histiocytic) and T lymphocytes in these tissues may exert some regulation over hematopoiesis.

The progenitors of the hematologic lineages will generate clonal colonies *in vitro* in semisolid media in response to specific cytokine combinations (*2–4*), hence the name colony-forming units (CFUs). The myeloid lineage contains the CFU-granulocyte/monocyte (CFU-GM) and the more mature progenitors, CFU-granulocyte and CFU-monocyte, which produce pure granulocyte or monocyte colonies, respectively (*5–7*). This lineage also contains progenitors for CFU-eosinophils or CFU-basophils. The erythroid lineage contains the CFU-erythroid, which forms hemoglobinized colonies in response to erythropoietin (*8–10*), and the burst-forming unit-erythroid (BFU-E), which produces large, multifocal colonies of hemoglobinized cells in response to erythropoietin plus cytokines with burst-promoting activity (*9,11–19*). The megakaryocytic lineage contains the CFU-megakaryocyte (CFU-Mk) (also called CFU-Meg) (*20–22*), which responds to many cytokines, including the c-*mpl* ligand (*23–30*). By analogy to BFU-E, several laboratories report a BFU-megakaryocyte, which forms large, multifocal colonies (*31,32*). The lymphoid lineages also contain progenitors that form clonal colonies of B or T cells when stimulated with interleukin(IL)-7 and IL-2, respectively (*33–35*).

In addition to these lineage-restricted progenitors, hematopoietic tissue contains immature progenitors that form colonies containing multiple myeloid lineages (*36–42*). One such progenitor is CFU-granulocyte, erythroid, megakaryocyte, and monocyte (CFU-GEMM). As the name implies, colonies formed by this pluripotent progenitor are distinguished by the presence of cellular elements from all of these lineages.

Several CFUs lie close to the hematopoietic stem cell developmentally. The high proliferative potential–colony-forming cell (HPP-CFC) produces a very large colony that contains primarily cells with a blastlike cytology, a small proportion of which will reform a clonal colony after

recloning (*43–50*). The long-term culture-initiating cell (LTC-IC) is a cell with multilineage potential found at low frequency in the marrow, probably positioned prior to the HPP-CFC in myelopoiesis (*51–54*). LTC-IC cells exhibit several characteristics of stem cells, including some capability of self-renewal, maintenance of both lymphopoiesis and myelopoiesis, and long-term reconstitution of a lethally irradiated host (*55–64*).

Bone Marrow Stroma

In contrast to the proliferation and expansion of tissue mass by hematopoietic cells, the primary function of the stroma is the nurture and support of developing blood cells (*52,65–67*). However, there is a stromal colony-forming unit called CFU-fibroblastoid (CFU-f), which produces a colony composed of adherent cells exhibiting morphologic features of fibroblasts, adipocytes, and other stromal elements (*65,68*). Although proliferating CFU-f is highly sensitive to many bone marrow toxicants *in vitro*, it is unclear whether the CFU-f is an *in vivo* target cell of toxicants that affect replicating cell types.

Interactive Cultures of Hematopoietic and Stromal Cells

Cultures of hematopoietic cells combined with stromal support cells can be maintained for several months in the absence of exogenously added cytokines by adhering to prescribed methodology (*69–72*). These so-called Dexter cultures sustain myelopoiesis at nearly steady-state levels for several weeks, after which time myeloid cell output begins to decline. It is possible to quantitate the output of progenitors or mature myeloid cells over time in these cultures. Likewise, long-term marrow cultures called Whitlock–Witte cultures sustain lymphopoiesis (*73–76*). Modifications of cell culture conditions can switch the cultures between lympho- and myelopoiesis, making it possible to evaluate both lympho- and myelopoiesis from the same cell culture (*56,77*). Because cytokines are not added to these cultures, they are thought to model closely the steady-state hematopoiesis that occurs *in vivo*. However, the progressive decline in mature cell output indicates the need to develop culture methodology for maintaining hematopoietic tissues in a homeostatic state.

Multidisciplinary Application of *in Vitro* Hematotoxicology

A great variety of toxicologic problems have been investigated using *in vitro* assays of hematopoiesis. The following examples have been selected to illustrate the wide-ranging usefulness of *in vitro* hematotoxicology. For more detailed discussions about study design and interpretation, see several recent reviews (*1,78–93*).

Toxicity to the Mature Blood Cell Compartment

Toxicity affecting primarily the mature, differentiated compartment usually manifests clinically as rapid-onset cytopenia. An example of this toxicity is phenylhydrazine-induced anemia caused by direct hemolysis of erythrocytes (*10*). However, hematologic toxicity following xenobiotic exposure can also manifest clinically as leukocytosis. Eosinophilia associated with exposure to IL-2, lithium, or a contaminant in over-the-counter preparations of L-tryptophan is due to toxicant-induced secretion of hematopoietic growth factors from mature blood cells (*94–101*). Xenobiotic-induced leukocytosis can also be due to abnormally high neutrophil or lymphocyte counts (*102–105*). CFU assays usually do not play a critical role in investigating this type of xenobiotic toxicity unless it cannot be explained by increased cytokine secretion.

Toxicity to the Progenitor Compartment

The mechanism of hematotoxicity most frequently and thoroughly studied *in vitro* is the acute effects of toxicant on marrow progenitors like CFU-GM (referred to as CFC-c in the older literature) and CFU-Mk. Toxicity is quantified from the number of surviving progenitors as a function of exposure level under maximally stimulatory cytokine concentrations.

Substances such as antineoplastics, biologic toxins, and ionizing radiation (*106–116*) destroy the rapidly dividing marrow progenitors, and a single exposure can result in an acute yet reversible neutropenia or thrombocytopenia 4 to 20 days later. A rapid repopulation of the progenitor compartment precedes recovery of peripheral counts by several days (*106,117–124*). The assumption of *in vitro* investigations of toxicity is accurate recapitulation of the *in vivo* toxicity in the *in vitro* model. In fact, *in vivo* toxicity to progenitors can be reproduced *in vitro* after direct exposure to pharmacologically relevant exposure levels of toxicants (*78,81,83,84,106,125–136*). Clinically achievable concentrations of myelosuppressive anti-human immunodeficiency virus nucleosides like azidothymidine (AZT) inhibit erythroid and myeloid progenitors (*137–143*) but the dideoxypurines, which do not cause anemia or neutropenia, do not (*144,145*). Clonogenic assays have also distinguished the relative contribution of each component of a multidrug regimen to therapy-related agranulocytosis (*131,132,146,147*).

Drug exposure levels that inhibit colony formation by approximately 50% do not cause neutropenia clinically (*129,148*). It is likely that the *in vitro* and *in vivo* data fail to correlate at these mildly toxic levels of exposure because the hyperplastic response of the marrow can compensate for this magnitude of progenitor loss. For example, there may not be any hematologic consequence from a 2-fold reduction in the frequency of CFU-GM if balanced by a 2-fold increase in marrow cellularity (no net gain in total CFU-GM). A direct correlation may exist between the decreases in clonogenic survival and absolute neutrophil counts only when toxicant levels cause such a severe lesion in the progenitor population that marrow hyperplasia cannot compensate.

In vitro assays can also be used to investigate cytopenias caused by noncytotoxic xenobiotics. For example, substances that reduce the number of differentiated blood cells produced per progenitor would be expected to decrease the size of the clonal colonies (the number of cells per colony), but not the number of colonies. Colonies that resemble CFU-GM colonies but are too small are named clusters. Thus, reduced CFU-GM colony formation accompanied by a shift in the distribution of colony size toward clusters argues against destruction of the progenitor. Trichothecene mycotoxins appear to shift the balance between proliferation and differentiation toward the latter (*113–115*). Interferons, the transforming growth factor beta (TBF-β) family, and tumor necrosis factors inhibit colony formation by CFU-GM, CFU-GEMM, and HPP-CFC (*149–156*). Experiments with TGF-βs indicate how subtle yet specific these effects on progenitors can be (*149–151,154–156*). The principles of predicting the parameters of neutropenia from *in vitro* data have not yet been established for this category of toxicants.

There is considerable confusion regarding a quantitative relationship between progenitor toxicity and acute anemia or thrombocytopenia. Megakaryocytopoiesis involves not only mitosis but also endoreplication, and it is unclear whether cell-cycle dependent toxicants are also endoreplication toxicants. For CFU-Mk assays, spontaneous colony formation occurs *in vitro* without

exogenously added cytokines; and colony formation is quantified based on a Δ value, i.e., the net increase in colony number due to cytokine stimulation. A cytokine-free control is included to quantify the number of colonies that form independently of added cytokine.

Toxicant-induced anemia is also difficult to predict from erythroid progenitor assays. Because erythrocytes circulate with a relatively long half-life, an acute toxicant-induced disruption of erythropoiesis is probably insufficient to cause anemia. For example, dipyrone inhibited not only CFU-GEMM and CFU-GM but also BFU-E in the presence of serum from a patient with drug-induced agranulocytosis and thrombocytopenia but not anemia (*157*). Also, ceftazidime is an equally potent inhibitor of BFU-E and CFU-GM, although it is clinically associated with agranulocytosis (*158*).

The most difficult hematotoxicity to predict with *in vitro* assays is the progressive loss of one or more blood cell lineages during chronic exposure to a toxicant: agranulocytosis, pancytopenia, and aplastic anemia. In some cases the toxicity leaves a permanent dysfunction, while in other cases the toxicity resolves after identification and removal of the toxicant. The distinction between irreversible aplasias like aplastic anemia and the progressive yet usually reversible aplasias like agranulocytosis is important for proper application of *in vitro* assays. In practice, toxicity may not be definable as reversible or irreversible if the toxic insult cannot be identified or if the degree of permanent damage is insufficient to cause permanent symptoms. In the cases of irreversible marrow damage after multiple toxicant exposures, it is often impossible to know whether toxicity would have occurred after a single exposure.

Progressive yet reversible xenobiotic-induced cytopenia generally indicates a direct effect of toxicant or metabolite on hematopoietic cell types. CFU-GM levels in patients with drug-induced agranulocytosis are depressed relative to controls (*91,159–164*), and inhibition of myeloid and erythroid progenitors is a likely mechanism for beta-lactam-induced agranulocytosis (*158*) and contributes to phenylhydrazine-induced anemia (*10*). In these cases the *in vitro* progenitor assays correctly identified the mechanism of myelosuppression. Hypersensitivity of progenitors from susceptible individuals to the toxicant or a toxic metabolite can be demonstrated with progenitor assays (*161,165–171*). However, other xenobiotics (chlorpropamide, phenytoin, methimazole, valproate, disopyramide, and phenacetin) not only inhibit progenitors directly but also induce both cellular and soluble immune mechanisms, which inhibit hematopoiesis (*161,163,172–176*). Because immune constituents can contribute to hematotoxicity, it is important to eliminate effector cells when trying to prove a direct toxicity of xenobiotic to progenitors *in vitro* (*168*). Otherwise, the effects of inhibitory cytokines released by mature T cells and monocytes in response to toxicant may be measured instead, which would be a toxicity to the mature blood cell compartment rather than the progenitor compartment. Unfortunately, in many cases when the immune system contributes to hematotoxicity, *in vitro* studies using marrow from normal donors are not informative and in fact are usually inconclusive (*167,170,177*).

It is encouraging that several laboratories arrived independently at similar assay conditions for quantifying toxicity to hematopoietic progenitors (*1,81–85,178,179*). Microculture techniques for progenitors are also available when xenobiotic is in short supply (*180*).

Toxicity to Bone Marrow Stroma

The microenvironment is a target for toxicant injury (*90*), and assays of stromal function have been used to investigate the effects of toxicant exposure. Bone marrow stroma is more sensitive than hematopoietic progenitors to the toxicity of acute neutron irradiation but less sensitive to X rays, and hematopoietic stem cell and stromal cell repopulation, but not progenitor survival, are dependent on dose rate (*109,112,124,181–184*). Long-term bone marrow cultures based on Dexter's method (*69*) have been used to investigate the agranulocytosis associated with ceftazidime and other agents (*82,158*). In addition, the CFU-f is potentially a dose-limiting cell type for radiation and some quinones of benzo[*a*]pyrene (*183–185*). CFU-f is the most sensitive progenitor to AZT toxicity, and AZT causes perturbations in parameters of long-term bone marrow assays at clinically relevant concentrations (*186*). Both conventional and high-dose cancer chemotherapies cause permanent damage to the marrow microenvironment (*111,148,187–192*). However, clinical hematology parameters can recover and even normalize in spite of continuing *in vitro* measurements of impaired marrow function (*148,182, 189–195*), casting doubt on the power of these *in vitro* assays to predict clinical outcome after chronic exposure.

Conceptual Framework for *in Vitro* Hematotoxicology Studies

Alternative hematotoxicology has benefited tremendously from the progress in experimental hematology over the past 40 years. Recombinant cytokines are available, and consequently a number of hematopoietic cell types can be assayed. *In vitro* hematotoxicology differs significantly from other alternative toxicologies in that the actual target cell of the toxicant can be studied in the laboratory. In addition, human exposure–hematotoxicity relationships are available from clinical trials in oncology for quantitative correlation with *in vitro* data. Finally, there appears to be little disagreement over the standardized conditions to use for quantitative assays and several laboratories in this field have reached similar conclusions about experimental variables via independent studies (*1,81,84,85,178,179*).

What Should Be Predicted?

Clinical hematotoxicity resulting from xenobiotic exposure can be described by four parameters (Figure 1): severity of the change in blood cell counts (cytopenia or cytosis), time from exposure to the most severe toxicity, duration of the nadir, and time required for recovery from the toxicity (degree of reversibility). These parameters completely describe the clinical course and nature of xenobiotic-induced cytosis and cytopenia (*88*). These toxicologic parameters have several important characteristics. They are not linked to any assumptions about molecular mechanism; they are clinical end points measured in patients and therefore they constitute the *in vivo* data that *in vitro* end points must predict, and they provide a quantifiable description of xenobiotic effects on human blood cell counts.

Because regulatory agencies must make decisions about human PELs even for compounds with unknown mechanisms of action, it is preferable to have mechanism-naive assays, i.e., assays predictive across broad mechanistic classes. Molecular mechanism is not trivialized by this approach; rather it is admitted that this information is not required to regulate the product or treat the intoxicated patient. For example, any compound that caused reversible thrombocytopenia of 10 days' duration will be treated similarly, whether the xenobiotic disrupted

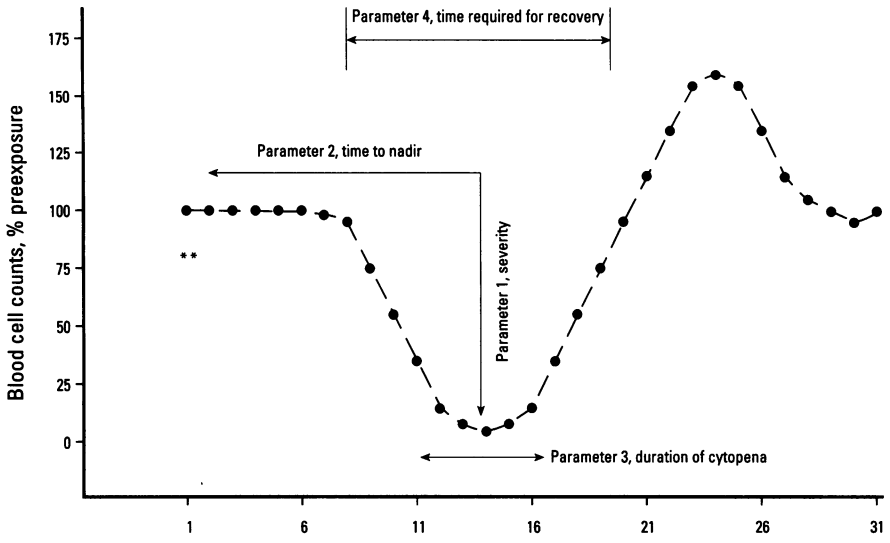

Figure 1. Four independent parameters describe acute hematologic toxicity *in vivo*. In leukocytosis, the parameters are modified to the maximum increase in leukocyte counts, time to the maximum, duration of the maximum, and time to recovery. *In vitro* hematotoxicology can be focused on the prediction of these four parameters in the human prior to any actual human exposure. Initial efforts have focused on validation of *in vitro* systems for predicting the severity of the neutrophil nadir after acute (preferably single dose) exposure. ** indicates the time of acute toxicant exposure.

signal transduction or destroyed progenitors. We view mechanism-independent toxicology as generally useful and advantageous both because it prevents assay proliferation syndrome, in which a new assay must be created for each mechanistic class that must be regulated, and because it can contribute to the regulation of new products with poorly understood mechanisms of action.

The goal of *in vitro* hematotoxicology is the prediction of these four parameters (*in vivo* end points) from end points obtained from assays of human hematopoietic function after *in vitro* exposure to the toxicant (*84,88*). Different *in vitro* end points, and perhaps even different hematopoietic assays, may be required to predict each *in vivo* parameter. Most studies focus on the prediction of the severity of the nadir.

Extending the Concept of Dose-Limiting Tissue to a Dose-Limiting Cell Type

Within any hematologic lineage there are a number of potential target cells for the toxicant: the mature cell compartment in the bloodstream, the progenitors in the marrow that make these blood cells, and the supporting cells in the hematopoietic tissues that regulate blood cell production. Most of these can be assessed for toxicity *in vitro* and it is important to determine which is the actual target cell of the toxicant *in vivo*. This is especially important because many toxicants may show some degree of *in vitro* toxicity to all of the cell types in the lineage but only the *in vitro* end point from the assay of the actual target cell population will be the most likely predictor of clinical toxicity. Given data from assays of mature blood cell lysis and dysfunction, progenitor survival and function, and stem cell and stromal cell function, how does one predict what will be the clinical manifestation of toxicant exposure?

It is a well-established principle of *in vivo* toxicology that the adverse effects of exposure are determined by the most sensitive of the exposed tissues. The most sensitive tissue is called the dose-limiting tissue, and *in vitro* toxicology must predict the ramifications of exposure in this tissue to predict clinical toxicity. By analogy, the manifestations of toxicant exposure in the dose-limiting tissue must be determined by its most sensitive cell compartment, which we have called the dose-limiting cell type (*88*). This analogy is helpful in the interpretation of *in vitro* data when a toxicant is evaluated on the entire spectrum of cell types that constitute a hematopoietic lineage. For example, the nature of the neutropenia caused by a toxicant will be determined in large part by whether mature neutrophils, immature neutrophils, myeloid progenitors, stem cells, or myelopoietic support cells are the *in vivo* target of the xenobiotic. Until more is understood from attempts at *in vitro–in vivo* correlations, it seems reasonable to assume that the dose-limiting cell compartment *in vivo* is the cell type most sensitive to the toxicant *in vitro*.

It is possible that by identifying the dose-limiting progenitor, we may predict the time to nadir. For the neutrophil lineage, this hypothesis postulates that neutropenia due to a lesion in an immature neutrophil progenitor population will take longer to appear than a lesion in a relative late-stage neutrophil progenitor pool. This has yet to be proven, however.

In Vitro Pharmacology

The *in vitro* assays are relatively simple to perform and a wealth of data can be generated in a short period. In general, the results of the test will be expressed as the change in an *in vitro* end point (Y axis) as a function of the level of toxicant exposure (X axis, usually in concentration units) using a clinically relevant duration of exposure (Figure 2A). The next step is to translate this *in vitro* data into clinical language and predict the clinical consequences of toxicant exposure. However, the way this goal has been phrased implies a subtle distinction from the conventional purpose of toxicology studies. Traditionally, the question is one of classification: Is this compound a hematotoxicant? However, it seems reasonable to assume that any toxicant will be a hematotoxicant if the other tissues of the body can tolerate high enough exposure levels. Certainly there will be compounds that inhibit CFU-GM *in vitro* but do not cause neutropenia, because exposure levels never reach a high enough level: for example, the nonmyelosuppressive nucleoside analog dideoxycytidine is in fact toxic to CFU-Mk and CFU-GEMM *in vitro* (*116,144,145*). Because toxicologic experience lends credibility to this assumption, the practice of classifying chemicals as hematotoxicants should be abandoned.

If classification is to be abandoned, what prediction should replace it? We have attempted to answer this question during the past 7 years. The PELs for most regulated products are based on the NOAEL dose, whereas antineoplastics are regulated based on the MTD. We have come to understand that the most useful *in vitro* end points are those that predict the level of systemic exposure to toxicants at these two doses of regulatory significance. Hence, the purpose of *in vitro* hematotoxicology is to predict the toxicant exposure levels associated with the MTD and the NOAEL doses. We have termed the predictive *in vitro* end points for these two exposure levels the

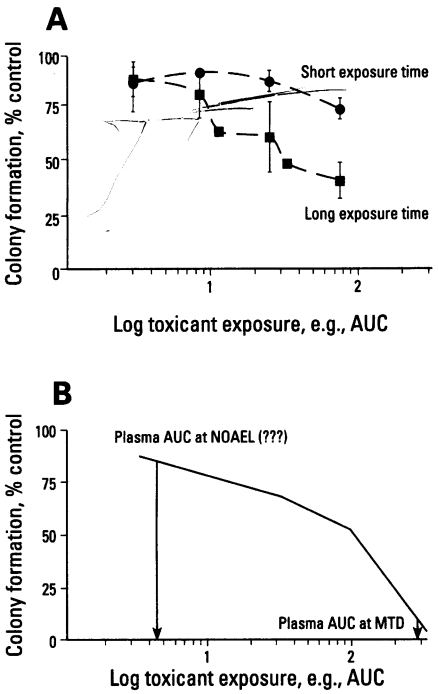

Figure 2. (*A*) Typical *in vitro* hematotoxicology data relating the decrease in a hematopoietic end point as a function of toxicant exposure in units of concentration, AUC, or whatever pharmacologic parameter of exposure is most closely linked to inhibition. The data shown here reflect the differential toxicity between a brief and long duration of exposure commonly observed with cell cycle-dependent toxicants. (*B*) *In vitro* toxicology attempts to relate a cell culture end point to a regulatory end point such as the NOAEL or the MTD. *In vitro* systems relate changes in end points to concentration-based measurements, and the estimation of dose relies on estimates of pharmacokinetic parameters. Several lines of evidence point to the AUC that inhibits CFU-GM by 90% (e.g., the AUC at IC_{90}) as the predictive end point for the plasma AUC that will cause grade 4 neutropenia at the MTD (Table 1). The *in vitro* end point that predicts the AUC at the NOAEL has not been determined.

MTC and NOAEC, respectively. This is analogous to the determination of the no effect and maximum tolerated doses *in vivo*, except that *in vitro* systems use concentration units instead.

For many hematotoxic compounds the severity of toxicity is related to a pharmacologic measure of exposure called the area under the plasma concentration versus time curve (AUC). Thus, the hypothesis of *in vitro* hematotoxicology is that the *in vitro* AUCs at the MTC and the NOAEC are identical to the plasma AUCs at the MTD and the NOAEL, respectively. This strategy shifts the emphasis of the *in vitro* study to end points that lie on the X axis, not the Y axis. We should not try to predict whether compounds inhibit neutrophil production: rather we should assume that they could, and determine the AUC at the NOAEL and MTD for the neutrophil lineage (Figure 2B).

This understanding clarified how to determine if the CFU-GM assay could be validated for predicting neutropenia and used in the regulatory setting (*88*). We concentrated exclusively on this hematotoxicity because the relationship between progenitor numbers and peripheral blood cell counts is simpler for neutropenia than for anemia or thrombocytopenia. Furthermore, to identify the principles and concepts to include in clinical prediction models, we restricted our initial studies to the simplest of toxicity, the reversible neutropenia caused by an acute toxicant exposure. This rationale also focused the ECVAM validation study in hematotoxicology on the prediction of neutropenia (*1*).

Predicting Toxicant Exposure (Plasma AUC) at the MTD from *in Vitro* Data

Because of our interest in the clinical development of antineoplastic agents and its regulation, most of our *in vitro–in vivo* correlation studies have focused on the identification of the *in vitro* end point that is the MTC (Table 1). A quantitative study of pyrazoloacridine found close correlation between inhibition of *in vitro* colony formation and the grade of neutropenia *in vivo* (*129*). In this study, the AUC that caused grade 3 to 4 neutropenia in humans inhibited *in vitro* colony formation of human CFU-GM by 90%. The result suggested that the 90% inhibition concentration (IC_{90}) from the human CFU-GM assay was the MTC when this progenitor is dose limiting. This conclusion was confirmed and extended in the mouse by showing that the AUC at the IC_{90} from the murine CFU-GM assay was associated with a 90% reduction not only in absolute neutrophil count but also marrow CFU-GM (R Parchment, unpublished data). Subsequently, the IC_{90} was the *in vitro* end point that predicted the differential between human and mouse MTD for the topoisomerase I inhibitor topotecan, whereas other IC end points failed to predict the MTD differential from the nonparallel human and mouse concentration–toxicity curves. Preliminary results show that the IC_{90} ratio correlates to the MTD ratio for seven of seven tested drugs. Current studies are examining whether the IC_{90} is the MTC for other progenitor compartments and for antineoplastic agents with other mechanisms of action. It is possible that a 90% reduction in content might not be tolerated in other progenitor compartments, while confirmation of this putative MTC for CFU-GM and additional toxicants will help clarify prediction models and gain regulatory acceptance. Based in part on these studies, the ECVAM validation study of the CFU-GM assay will attempt to validate the IC_{90} as the MTC from which the plasma AUC at MTD can be predicted for xenobiotics associated with dose-limiting neutropenia (*1*).

Predicting Toxicant Exposure (AUC) at the NOAEL from *in Vitro* Data

Although the NOAEL is used much more frequently than the MTD as the basis for setting PELs for regulated products, there is a lack of understanding about the maximum degree of loss of progenitors that does not result in clinical neutropenia. This issue directly relates to our understanding of the resilience of hematopoietic tissue to toxic insult and the compensatory mechanisms, such as marrow hyperplasia, that can overcome mild reductions in progenitor survival. For example, a 50% reduction in CFU-GM survival coupled to a 2-fold increase in marrow cellularity would not result in any net loss of neutrophil progenitors. During our *in vitro–in vivo* correlation study in the Phase I trial of 9-methoxypyrazoloacridine (*129*), the greatest plasma AUC that did not cause neutropenia inhibited human CFU-GM by 35%. Although circumstantial, these results suggest that the IC_{35} may be the NOAEC, i.e., the end point from which the AUC at NOAEL can be predicted. Unfortunately, the minimum number of progenitors required to maintain normal peripheral blood cell counts *in vivo* has not yet been determined for any myeloid lineage in any species. Until the IC_{35} is established as the *in vitro* NOAEC, the IC_0 or perhaps IC_{05} should be used to estimate the NOAEL *in vivo* (*1*). Obviously, there is a pressing research need to determine the exact relationship between progenitor numbers and peripheral blood cell counts in each of the myeloid lineages and then identify the most predictive IC value to use for each level of myelosuppression *in vivo*. We are currently examining other compounds to determine if the IC_{35} consistently predicts the AUC at the NOAEL.

The Critical Importance of Identifying the MTC and the NOAEC

Knowing the NOAEC and the MTC simplifies what otherwise would be very

Table 1. Identification of the IC_{90} as the MTC in CFU-GM assays.

Toxicant	Species	Evidence	Reference
9-Methoxypyrazoloacidine	Human	*In vitro–in vivo* correlation with ANC	Parchment et al. (*129*)
	Mouse	Intentional exposure at $IAUC_{90}$ produced 85% reduction in ANC	Parchment et al. (*129*)
Topotecan	Human and mouse	Nine-fold differential for both MTD and IC_{90}, but not for IC_{50}	Erickson-Miller et al. (*219*)
Flavopiridol	Human and rat	Rat more sensitive than human at IC_{90}, but more resistant at IC_{50}, and rat MTD lower than human MTD	J Tomaszewski (unpublished data)
Fludarabine, fazarabine, camptothecins, amonafide	Multiple species	IC_{90} ratios and MTD ratios correlate	J Tomaszewski (unpublished data)

Abbreviations: ANC, absolute neutrophil count; $IAUC_{90}$, AUC at the IC_{90}. The toxicants are antineoplastic agents that cause myelosuppression; these are useful test compounds because neutropenia is an acceptable risk of human exposure.

complicated data interpretation (*87,88*). For example, it is common practice to compare a new compound with a reference compound of known hematotoxicity *in vitro*. Suppose one obtains data from such a study that shows a crossing of the concentration–response curves and it cannot be explained by differential chemical stability (Figure 3). From these data, can one predict whether humans can tolerate higher, the same, or lower levels of the investigational compound than the reference compound? In other words, should the PEL be higher or lower than the reference compound? If one knows that the IC_{90} is the MTC and that grade 3 to 4 neutropenia is an acceptable risk of human exposure (e.g., a cancer drug), then clearly the tolerated AUC is higher for the investigational product than for the reference compound. Assuming equivalent pharmacokinetics, the PEL can then be set higher for the new product than for the reference product. In contrast, if the IC_{35} is the NOAEC and neutropenia is not an acceptable consequence of exposure to this product, the opposite conclusion would be reached. The PEL for the investigational product should be set lower than the reference product because its IC_{35} is lower. This theoretical example illustrates how the interpretation of the *in vitro* data depends on the intended use of the product. For one use the PEL can be higher, whereas for another use it must be lower. This example also suggests how to choose a least hematotoxic analog from *in vitro* data when the analogs do not exhibit parallel curves. The least toxic analog is that with the least inhibition in an appropriate *in vitro* assay at the end point most relevant to the product's intended use. Such data would be impossible to interpret without these X-axis concepts.

The identical issue arises when using *in vitro* hematotoxicology to directly compare human toxicant sensitivity to that of the preclinical animal species in which the product is tested *in vivo*. In this case, the question is whether the preclinical toxicology species under- or overestimates human PELs for myelosuppressive compounds. If the direct comparison results in parallel curves (Figure 4A) then this question is easily answered. The human:animal ratio at any IC value will provide an estimate of the difference in tolerance of the compound; and, because the IC_{50} is the most accurately determined point on these curves, the IC_{50} ratios should be used. However, consider the more typical case of nonparallel curves across species (Figure 4B). Without knowing which IC values to compare, it would be difficult to interpret these data and predict human tolerance. However, a comparison of the IC_{90} end point across species shows that humans can tolerate more than dogs but less than rodents. Assuming there are no pharmacokinetic differences across species, the MTD differential will be proportional to the IC_{90} differential. In contrast, if neutropenia is an unacceptable risk of exposure, the NOAEC will be used for comparison. Assuming the IC_{35} is the NOAEC, one would predict that the human PEL could be set higher than the rodent PEL by an amount proportional to the *in vitro* differential in the NOAECs.

Clinical Prediction Models for Prospective Evaluation and Validation Trials

Several prediction models have been developed that use the MTC and the NOAEC as predictive *in vitro* end points for the plasma AUC at the MTD and the NOAEL, respectively. From this estimate and knowledge of human pharmacokinetics

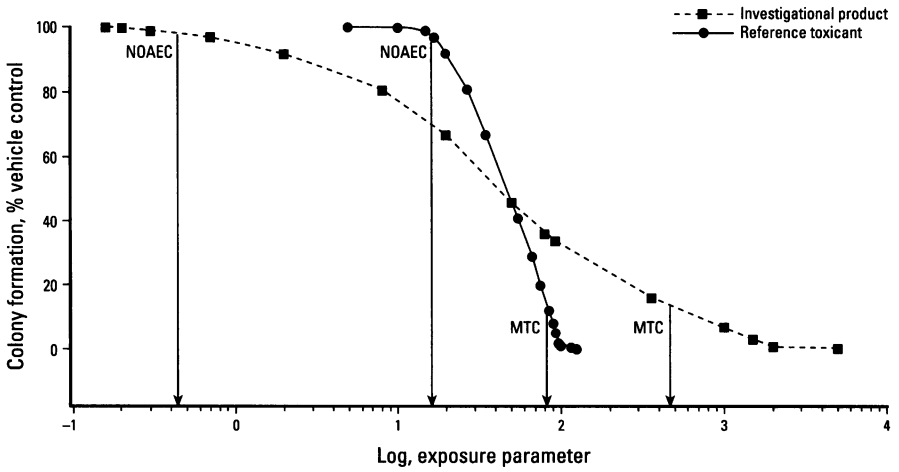

Figure 3. Differences between the test article and the reference compound depend on the level of inhibition at which the comparison is made. Knowing which *in vitro* end points are the NOAEC and the MTC makes it possible to interpret such *in vitro* hematotoxicology results. Note that the NOAEC is lower for the new product than the reference toxicant; in contrast, its MTC is higher. This interpretation assumes neutropenia is the dose limiting toxicity for both compounds.

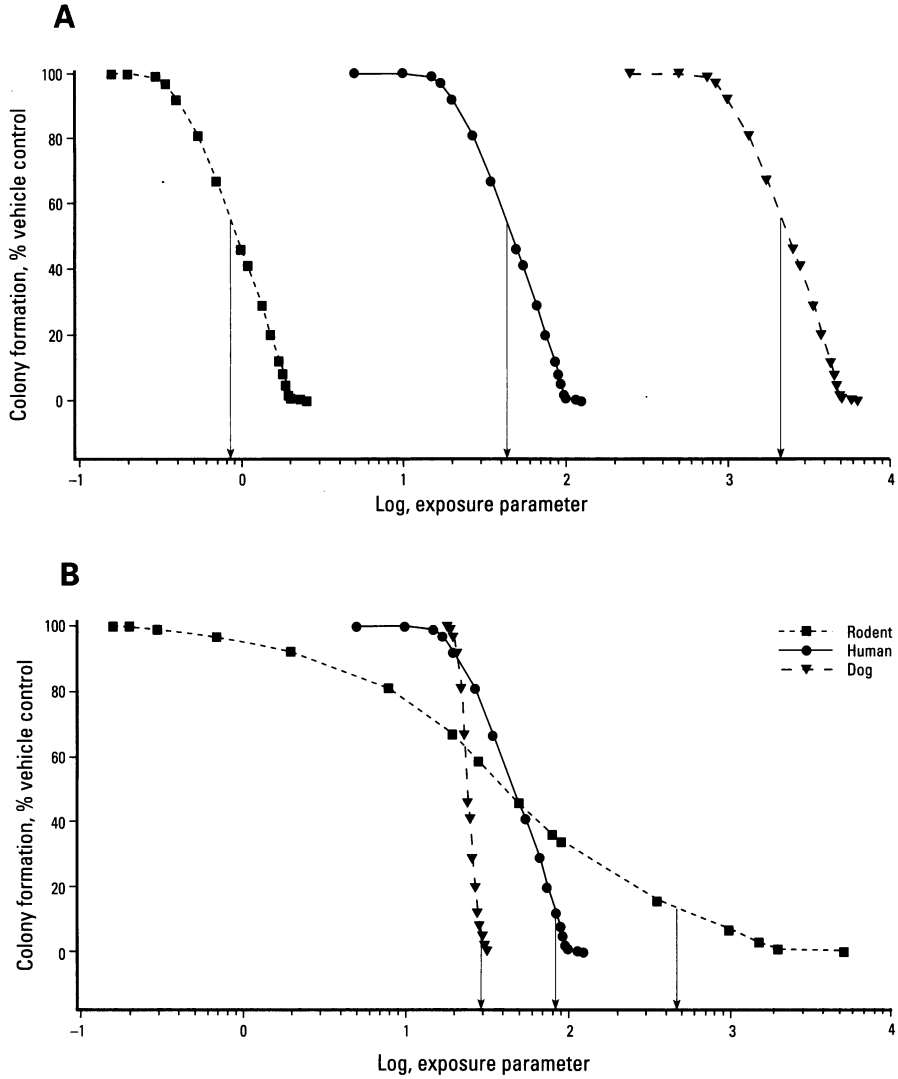

Figure 4. (*A*) In the ideal situation, comparative toxicology data across species will map to parallel curves so that differentials in toxicant tolerance can be based on the IC_{50} values for two reasons: the ratio of any IC value will be the same and the IC_{50} is the most accurate point using conventional curve fitting methods. (*B*) In many cases data from multiple species must be compared across nonparallel curves that may even cross each other over the tested concentration range. In this case, knowing which *in vitro* end points are the NOAEC and the MTC is critical for data interpretation and clinical prediction. For example, these data predict that the MTD will be higher in rats, humans, and then dogs, assuming neutropenia is the dose-limiting toxicity and the IC_{90} is the MTC. In contrast, the data predict that the NOAEL will be highest in humans, followed by dogs and then rats, assuming the IC_{35} is the NOAEC.

and plasma protein drug binding, the doses that achieve these AUCs in the plasma of exposed humans can be determined. However, the models can only be applied to compounds that are expected to show dose-limiting neutropenia. This is a critical assumption for *in vitro* hematotoxicology, and *in vivo* toxicology studies must indicate that neutrophil precursors are the dose-limiting target. If the dose-limiting toxicity of the investigational product was e.g., cardiotoxicity, determination of AUC at the MTC for CFU-GM would overestimate human tolerance and jeopardize patient safety. Some studies have used the IC_{70} instead of the IC_{90} as the MTC, and these two end points will be compared during some of the ongoing studies. The IC_{70} may be more appropriate when estimating PELs for products that will be used in patients with impaired marrow function. Prospective evaluation of these models will be critical in gaining acceptance, and Phase I clinical trials (dose-escalation trials) of antineoplastic agents provide an excellent opportunity for such an evaluation. The following models focus on the prediction of MTD; prediction of the NOAEL would simply substitute the IC_{35} or other NOAEC end point for the IC_{90}.

Prediction Model 1 is the simplist model. It can be used if human pharmacokinetic parameters are unknown or cannot be determined. Model 1 has the greatest level of uncertainty because it incorporates only pharmacodynamics. It is based on the idea that neutrophil progenitors can serve as a sentinel tissue for interspecies comparisons. Large interspecies differences in toxicant disposition (AUC as a function of dose) could lead to significant errors in the predicted MTD. Step 1: determine the MTD in an animal model; step 2: determine the toxicity differential between the animal and human dose-limiting progenitor based on IC_{90} values; step 3: adjust the animal MTD for the IC_{90} differential; and step 4: adjust the MTD again for differences in free drug concentration between the animal and human (e.g., protein binding).

Prediction Model 2 can be used when the AUC cannot be measured at the IC_{90} for the human progenitor but human pharmacokinetic parameters are known or can be predicted. This model assumes the marrow toxicant causes an AUC-dependent cytotoxicity. The model adjusts the plasma AUC at the MTD in the animal studies for species differentials in drug tolerance and plasma protein drug binding. Step 1: determine the plasma AUC at the MTD in an animal model; step 2: determine the toxicity differential between the animal and human dose-limiting progenitor based on IC_{90} values; step 3: adjust the animal-derived plasma AUC by the IC_{90} differential; step 4: adjust the plasma AUC for differences in free drug concentration between species; and step 5: using human pharmacokinetic parameters or estimated parameters, calculate the dose that gives the predicted AUC.

Prediction Model 3 aims to predict the actual human MTD for the neutrophil lineage. It should be the most accurate prediction model because it incorporates both human pharmacodynamics and pharmacokinetics. Step 1: identify the most sensitive neutrophil progenitor in human bone marrow; step 2: determine the IC_{90} for the dose-limiting human progenitor; step 3: derive the *in vitro* AUC at the IC_{90} by integrating the $C \times t$ curve; step 4: translate the *in vitro* AUC into *in vivo* terms by adjusting for differences in free concentration under the two conditions of exposure (e.g., protein binding);

and step 5: using human pharmacokinetic parameters or estimated parameters, calculate the dose that gives the predicted AUC.

Summary

Although our knowledge of how to use *in vitro* hematotoxicity data is in its infancy, these examples illustrate the progress that has been made by assessing exactly what end point is needed, i.e., what clinical end point should be predicted. The realization that X-axis rather than Y-axis end points are required for prediction was in a sense a breakthrough that has made it possible to propose clinical prediction models for prospective evaluation and validation (*1,88,129*). A second important breakthrough was our realization that predicting neutropenia actually involves the prediction of four independent parameters that, when taken together, describe clinical neutropenia: severity at nadir, time to nadir, duration of nadir, and time to recovery (*84,88*). Progress to date has been exclusively in the prediction of the severity of neutropenia (*88,129*). We are not aware that any progress that has been made in predicting the other parameters from *in vitro* data. Therefore, assays for CFU-GM and other granulocyte progenitors can be considered useful for investigating the cellular mechanism underlying the severity of reversible neutropenia and for determining the level of toxicant exposure that will be associated with grade 3 to 4 neutropenia. The investigation of the other parameters of neutropenia with *in vitro* assays should be considered exploratory research rather than established testing methodology.

Detailed Planning of *in Vitro* Hematotoxicology Studies

This section briefly covers some of the details that must be addressed when planning an *in vitro* hematotoxicology study (*84,87,88*).

Analytical Chemistry

There is substantial variability in the stability of different products under the conditions of the *in vitro* bone marrow assay, and subtle changes in the culture conditions can dramatically alter *in vitro* bioavailability. For example, many camptothecins show large differences in stability as a function of the species from which the culture medium serum or albumin is derived (*196–200*). These data show that one cannot assume that chemically related compounds have identical protein binding or stability *in vitro* and *in vivo* (*84,88*). In addition, some toxicants bind extensively to the cell culture containers (*201*). Therefore, the absolute $C \times t$ curve of a test substance at the MTC and the NOAEC under the conditions of the bone marrow assay must be determined chemically to determine the AUC. Chemical analysis also provides dose confirmation. Highly misleading results can be obtained when comparing two compounds, one of which is chemically stable and the other unstable, or one that is highly soluble versus one that is partially soluble. Unstable compounds and poorly soluble compounds will appear less toxic than equally potent stable or soluble compounds simply because the former have lower *in vitro* bioavailability than the latter. Concluding from *in vitro* data that a compound is relatively nontoxic, when in fact the data reflect an artifactual rapid decomposition of compound in the culture medium, could cause serious underestimation of clinical hematotoxicity. Thus, colony inhibition data must be accompanied by sound analytical data before one can make clinical predictions, and analyte quantitation is an important aspect of the ECVAM validation study of the CFU-GM assay (*1*).

Duration of Exposure

In vitro data should be obtained not only for relevant concentrations but also for relevant durations of exposure. The duration of *in vitro* exposure to each test substance should mimic as closely as possible the duration of *in vivo* exposure. For example, if *in vivo* exposure is multiday, brief *in vitro* exposures are unnecessary. Likewise, if the substance rapidly decomposes *in vitro* then only brief *in vitro* exposures should be used. In fact, prolonged *in vitro* exposures to unstable compounds expose the cells to potentially myelotoxic breakdown products that may not be present *in vivo*. The effects of decomposition on perceived toxicity become magnified as the time of compound exposure becomes much larger than the time of decomposition.

When toxicant exposure is brief, it must be decided whether to preexpose the hematopoietic cells to cytokine, stimulating their entry into the cell cycle, or to expose the hematopoietic cells immediately after isolation from the human tissue source. Toxicants that show cell cycle-dependent toxicity would be expected to show significant differences in toxicity between the two conditions. This is an important question of experimental design that needs exploration.

Endogenous (Naturally Occurring) Antagonists and Protein Binding of Toxicants

For maximized correlation between *in vitro* and *in vivo* data, the *in vitro* assays must take into account physiologic levels of endogenous biochemicals that influence the toxicity of the test compound. Obvious examples of endogenous antagonists include pyrimidines and purines and their nucleosides for toxicants that target nucleoside and nucleic acid metabolism, and glutathione and other nucleophiles for alkylating agents. There are also cotoxicants such as molecular oxygen for free-radical generating compounds. The concentrations of both antagonist and cotoxicants must be as close as possible to *in vivo* levels.

The plasma proteins that bind xenobiotics (albumin, α_1-acid glycoprotein) are another class of substances endogenous to both the *in vitro* and *in vivo* testing environments that introduce another source of error during interpretation of *in vitro* data. One cannot assume that a particular compound will behave identically in plasma from different species (such as from humans *in vivo* but from bovine or equine sources for *in vitro* assays), and the problem is especially significant for compounds that circulate tightly bound to carrier proteins. The levels of these proteins in the culture medium can dramatically affect the apparent potency of the test substance via changes in free (unbound) concentration of the actual toxicant (*84,88,196–203*). However, it is not necessary to add specific plasma proteins to the culture medium for *in vitro* hematotoxicity assays because results can be corrected for plasma protein binding measured in other simpler *in vitro* systems (*1*). It is best to compare several toxicants under identical culture conditions to learn about intrinsic differences in end-organ sensitivity and then correct the *in vitro* data for differences in protein binding determined in other assays. To facilitate *in vivo* extrapolation of *in vitro* data, the concentration–inhibition curve from bone marrow assays should express percent of inhibition as a function of free concentration of xenobiotic in the medium rather than total concentration. From these curves plus measurements of free versus total concentrations in human or animal plasma, it is possible to make *in vivo* predictions from *in vitro* data.

Protoxicants and Metabolic Activation

Although many toxicants are inactivated by metabolism, some compounds are

metabolized to toxic species. Human bone marrow stroma contains cytochrome P450s (CYP450s) that can bioactivate several toxicants, including polycyclic aromatic hydrocarbons, benzene, and cyclophosphamide (*92,204–210*). However, the metabolic capacity of the marrow stroma is small compared to liver, and it cannot produce sufficient levels of metabolite to reliably detect toxicity in the *in vitro* assays (*1,92*). Several *in vitro* systems are available for producing human CYP450 metabolites: liver microsomes, liver slices, hepatocytes, and transgenic cell lines expressing particular CYP450 isozymes. Hepatocytes or transgenic cell lines expressing specific CYP450s can be cocultured in the *in vitro* assays as a physically separate cell layer (*92*). Alternatively, stable metabolites can be isolated and evaluated individually in the *in vitro* assays.

The ECVAM validation study addresses several issues related to the selection of metabolizing systems and the *in vitro* study of protoxicants (*1*). S-9 fractions, dog liver microsomes, and rat liver microsomes are commonly used to produce metabolites. However, analysis of putative hematotoxic metabolites should use species-specific sources of metabolizing enzymes, i.e., human microsomes on human bone marrow or rat microsomes on rat marrow, because of the potential for interspecies differences in bioactivation (e.g., iododoxorubicin (*211*)]. Xenogeneic combinations are sometimes warranted to prove the species-specific nature of certain toxicology problems or the lack of relevance of animal toxicology findings to humans. In addition, to exclude any contribution of metabolites to hematotoxicity, they should be tested by coexposure with the parent compound to identify any synergism or antagonism that might affect hematotoxicity.

Sources of Human Hematopoietic Cells

Mononuclear cells and subpopulations like $CD34^+$ cells can be isolated from various sources: remnant marrow from femur or rib after surgeries, iliac crest aspirates from volunteers, umbilical cord blood, and peripheral blood leukocyte preparations (*31,32,39,48,53,64,70,117,150,155,178, 179,212–216*). Bone marrow-derived progenitors might be the most applicable to human toxicology, but cord blood-derived cells are more readily available for research in many countries. Pharmacologic differences, if they exist, between hematopoietic cells in adults versus cord blood may be most pronounced after exposure to cell cycle-dependent toxicants because these populations of cells show substantial differences in cell cycle status after isolation (*217*). One study that directly compared the chemosensitivity of human marrow and cord blood CFU-GM did not find any differences (*218*). In contrast, there are differences between marrow and cord blood progenitors and between mobilized and stationary progenitors in terms of autocrine cytokine stimulation, cell cycle status, and susceptibility to cytomegalovirus toxicity (*150,217*). Differences in cell cycle status between cord blood and bone marrow hematopoietic cells accentuate the need to determine whether preexposure to cytokines to stimulate entry into the cell cycle affects the predictive accuracy of *in vitro* models that use short duration of exposure.

Thoughts on the Immediate Future of *in Vitro* Hematotoxicology

Staying Current with Experimental Hematology

Experimental hematology is identifying hematopoietic cell types faster than toxicologists can determine if they are targets of toxicants. It cannot be assumed a priori that any progenitor is a target of hematotoxicants, but one cannot afford to assume that newly identified cell types are not targets and then be wrong. An efficient strategy to determine which of these cell types are targets is simply to determine which ones show altered levels in toxicant-exposed animals or patients with a time course that is consistent with a direct toxicant effect. Furthermore, a new progenitor population in a preclinical species that is a bona fide toxicant target but is not found in human marrow would create a serious toxicologic problem.

If mechanism-naive assays are advantageous in regulatory science, it is important to work toward the replacement of clonogenic assays with new assays that measure the rate of production of mature blood cells by hematopoietic tissues rather than by survival of progenitors. Kinetic measurements of cell output by toxicant-exposed progenitors would offer some advantages over clonogenic assays. First, the capability of the marrow to compensate for the loss of up to 30 to 40% of its progenitors via a hyperplastic response would be detectable. Second, initial rates of mature cell output by the exposed progenitor population could be analyzed sooner than colony formation (3–4 days vs 14 days), so efficiency and through-put would be increased. Third, assays that could quantify toxicant effects on the kinetics of mature cell production have the capability of detecting myelosuppression regardless of whether it is caused by cytotoxicity. Such an effect would be hard to detect and quantify using clonogenic assays (smaller colonies but no reduction in their number).

Nuances in Data Interpretation

Colony-forming assays contain additional information to colony size. One of these was discussed above: the toxicant that causes smaller colonies but does not decrease their number. Consider this question: If the result of compound exposure is to inhibit the development of the erythroid component in CFU-GEMM colonies, should this be scored as inhibition of CFU-GEMM (which it probably is not) or as inhibition of an early erythroid progenitor generated by the CFU-GEMM? If the latter, would percent inhibition of BFU-E give the same estimate of myelotoxicity as the percent reduction in the CFU-GEMM that contains erythroid elements? In the design of studies to evaluate these toxicants, it may be important to limit the duration of exposure to only a few hours so that lineage-restricted progeny generated by surviving CFU-GEMM are not exposed to toxicant. Prolonged exposures would confuse toxicity to CFU-GEMM with toxicity to the progeny of CFU-GEMM.

Predicting the NOAEL raises the issue of the definition of NOAEL. Should the NOAEL be defined only by a lack of clinical symptoms and pathologic changes or should it indicate a lack of cellular and molecular changes as well? Defining the NOAEL with ever more sensitive end points, from tissue to cell to molecular levels, does not account for the compensatory mechanisms that may provide for continued health in spite of constant exposure to xenobiotics. Should PELs for a xenobiotic be based on altered gene expression in the absence of any adverse clinical effects? Rather, should it be concluded that the indicator gene, although a marker of exposure, must have some threshold yet to be exceeded and that the change does not indicate risk? The definition of NOAEL is important in regulatory science, especially as technology becomes more sensitive at detecting minute changes and more facile at expedited quests for effects. These issues must be integrated into validation studies of the NOAEC for predicting the human NOAEL.

Recommendations for Research Support

Federal agencies can take the lead in accomplishing the following goals that will promote the validation, clinical acceptance, and regulatory usefulness of *in vitro* hematotoxicology:

- After the ECVAM study (*1*) validates the human CFU-GM assay for predicting the toxicant exposure level that causes grade 3 to 4 neutropenia, examine assays for the differentiated spectrum of myeloid progenitors in the marrow for predicting time to neutrophil nadir.
- Fund an evaluation of possible *in vitro* assays and end points to predict the duration of the neutrophil nadir and/or the time to neutrophil recovery. Currently, it is much easier to investigate marrow toxicity than marrow recovery. For example, there are no *in vitro* assays for predicting the clinical efficacy of cytokines used to stimulate hematopoietic recovery. Research is needed to know how to predict time to recovery, which is likely related to predicting irreversible hematotoxicity as well.
- Coordinate regulatory- and industry-based surveys of human bone marrow and cord blood uses and needs for *in vitro* hematotoxicology and propose solutions to obstacles that currently limit the use of human bone marrow in these assays, which is the actual human target tissue.
- Identify the NOAEC end point in the CFU-GM assay and prove that CFU-GM but not neutrophil counts decrease by this amount *in vivo* at this exposure level.
- Fund veterinary and clinical research to identify what progenitor stage(s) in the platelet and red blood cell lineages fluctuates in concert with peripheral blood platelet and erythrocyte levels.
- Fund an evaluation of all newly identified hematopoietic cell types defined by *in vitro* assays and determine if acute *in vivo* exposure to hematotoxic xenobiotics causes a decrease in their bone marrow levels, i.e., whether these newly identified cell populations are frequent targets for xenobiotics or not.
- Fund research into the replacement of clonogenic assays with new *in vitro* assays that quantify the rate of production of mature blood cells by toxicant-exposed hematopoietic cells.

Significance of *in Vitro* Hematotoxicology for Other Alternative Toxicologies

There are substantial ethical, political, financial, and regulatory pressures to replace and refine animal toxicology with alternative assays. Studies of the toxicant on its actual human target tissues would seem to be the best alternative. *In vitro* hematotoxicology does this, and as a result shows much promise for predicting clinical hematotoxicity, especially neutropenia caused by acute exposures. It provides an opportunity to obtain human toxicology and pharmacology information in the laboratory setting and an experimental basis for selecting an animal model for investigating clinical hematotoxicity.

The *in vitro* assays of xenobiotic effects on human hematopoiesis can be viewed as prototypes of future *in vitro* toxicology assays that will reveal concepts and principles of clinical prediction. The hierarchical structure of stem cells and progenitors in the hematopoietic system likely reflects similar hierarchies in other renewing tissues of the body, such as the gastrointestinal mucosa. The emerging principles for prediction described in this paper will be applicable to toxicity in these other renewing tissues once clonogenic assays for epithelial progenitors are developed and the colony-stimulating factors are available. However, it seems unlikely that what is learned in predicting hematologic toxicity will be of much help in predicting toxicity to nonproliferative tissues such as the nervous system; other end points and principles of clinical prediction will be needed for these more troublesome toxicities.

REFERENCES AND NOTES

1. Gribaldo L, Bueren J, Deldar A, Hokland P, Meredith C, Moneta D, Monesso P, Parchment R, Parent-Massin D, Pessina A et al. The use of *in vitro* systems for evaluating hematotoxicity. ATLA 24:211–231 (1996).
2. Wu AM, Siminovitch L, Till JE, McCulloch EA. Evidence for a relationship between mouse hemopoietic stem cells and cells forming colonies in culture. Proc Natl Acad Sci USA 59:1209–1215 (1968).
3. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells *in vitro*. J Cell Physiol 91:335–344 (1977).
4. Till JE, Price GB, Mak TW, McCulloch EA. Regulation of blood cell differentiation. Fed Proc 34:2279–2284 (1975).
5. Pluznik DH, Sachs L. The cloning of normal "mast" cells in tissue culture. J Cell Comp Physiol 66:319–324 (1965).
6. Bradley TR, Metcalf D. The growth of mouse bone marrow cells *in vitro*. Aust J Exp Biol Med Sci 44:287–299 (1966).
7. Ichikawa Y, Pluznik DH, Sachs L. *In vitro* control of the development of macrophage and granulocyte colonies. Proc Natl Acad Sci USA 56:488–495 (1966).
8. Stephenson JR, Axelrad AA, McLeod DI, Schreeve MM. Induction of colonies of hemoglobin-synthesizing cells by erythropoietin *in vitro*. Proc Natl Acad Sci USA 68:1542–1546 (1971).
9. Iscove NN, Sieber F. Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture. Exp Hematol 3:32–43 (1975).
10. Hara H, Ogawa M. Erthropoietic precursors in mice with phenylhydrazine-induced anemia. Am J Hematol 1:453–458 (1975).
11. Gregory CJ, Eaves AC. Human marrow cells capable of erythropoietic differentiation *in vitro*: definition of three erythroid colony responses. Blood 49:855–864 (1977).
12. Clarke BJ, Housman D. Characterization of an erythroid precursor cell of high proliferative capacity in normal human peripheral blood. Proc Natl Acad Sci USA 74:1105–1109 (1977).
13. Johnson GR, Metcalf D. Nature of cells forming erythroid colonies in agar after stimulation by spleen conditioned medium. J Cell Physiol 94:243–252 (1978).
14. Koury MJ, Kost TA, Hankins WD, Krantz SB. Response of erythroid day 3 burst-forming units to endotoxin and erythropoietin. Proc Soc Exp Biol Med 162:275–280 (1979).
15. Eliason JF, Van Zant G, Goldwasser E. The relationship of hemoglobin synthesis to erythroid colony and burst formation. Blood 53:935–945 (1979).
16. Wagemaker G, Peters MF, Bol SJ. Induction of erythropoietin responsiveness *in vitro* by a distinct population of bone marrow cells. Cell Tissue Kinet 12:521–537 (1979).
17. Meytes D, Ortega JA, Shore NA, Dukes PP. Human erythroid burst-promoting activity produced by phytohemagglutinin-stimulated, radioresistant peripheral blood mononuclear cells. Blood 54:1050–1057 (1979).
18. Nathan DG, Chess L, Hillman DG, Clarke B, Breard J, Merler E, Housman DE. Human erythroid burst-forming unit: T-cell

requirement for proliferation *in vitro*. J Exp Med 147:324–339 (1978).
19. Sonoda Y, Maekawa T, Kuzuyama Y, Clark SC, Abe T. Human interleukin-9 supports formation of a subpopulation of erythroid bursts that are responsive to interleukin-3. Am J Hematol 41:84–91 (1992).
20. Metcalf D, MacDonald HR, Odartchenko N, Sordat B. Growth of mouse megakaryocyte colonies *in vitro*. Proc Natl Acad Sci USA 72:1744–1748 (1975).
21. Nakeff A, Daniels-McQueen S. *In vitro* colony assay for a new class of megakaryocyte precursor: colony-forming unit megakaryocyte (CFU-M). Proc Soc Exp Biol Med 151:587–590 (1976).
22. Vainchenker W, Bouguet J, Guichard J, Breton-Gorius J. Megakaryocyte colony formation from human bone marrow precursors. Blood 54:940–945 (1979).
23. Williams N, Jackson H, Sheridan AP, Murphy MJ Jr, Elste A, Moore MA. Regulation of megakaryopoiesis in long-term murine bone marrow cultures. Blood 51:245–255 (1978).
24. Mei RL, Burstein SA. Megakaryocytic maturation in murine long-term bone marrow culture: role of interleukin-6. Blood 78:1438–1447 (1991).
25. Erickson-Miller CL, Ji H, Parchment RE, Murphy MJ Jr. Megakaryocyte colony-stimulating factor (Meg-CSF) is a unique cytokine specific for the megakaryocyte lineage. Br J Haematol 84:197–203 (1993).
26. Ogata K, Erickson-Miller CL, Nomura T, Abe K, Zhang Z, Murphy MJ Jr. Effects of recombinant cytokines on murine megakaryocyte colony formation in a serum-free fibrin clot culture system. Pathobiology 60:143–148 (1992).
27. Kaushansky K, Lok S, Holly RD, Broudy VC, Lin N, Bailey MC, Forstrom JW, Buddle MM, Oort PJ, Hagen FS et al. Promotion of megakaryocyte progenitor expansion and differentiation by the c-*mpl* ligand thrombopoietin. Nature 369:568–571 (1994).
28. Kaushansky K. The *mpl* ligand: molecular and cellular biology of the critical regulator of megakaryocyte development. Stem Cells (Dayt) 12(Suppl 1):91–96 (1994).
29. Broudy VC, Lin NL, Kaushansky K. Thrombopoietin (c-*mpl* ligand) acts synergistically with erythropoietin, stem cell factor, and interleukin-11 to enhance murine megakaryocyte colony growth and increases megakaryocyte ploidy *in vitro*. Blood 85:1719–1726 (1995).
30. Methia N, Louache F, Vainchenker W, Wendling F. Oligodeoxynucleotides antisense to the proto-oncogene c-*mpl* specifically inhibit *in vitro* megakaryocytopoiesis. Blood 82:1395–1401 (1993).
31. Nishihira H, Toyoda Y, Miyazaki H, Kigasawa H, Ohsaki E. Growth of macroscopic human megakaryocyte colonies from cord blood in culture with recombinant human thrombopoietin (c-*mpl* ligand) and the effects of gestational age on frequency of colonies. Br J Haematol 92:23–28 (1996).
32. Zauli G, Vitale L, Brunelli MA, Bagnara GP. Prevalence of the primitive megakaryocyte progenitors (BFU-meg) in adult human peripheral blood. Exp Hematol 20:850–854 (1992).
33. Choi KW, Bloom AD. Cloning human lymphocytes *in vitro*. Nature 227:171–173 (1970).
34. Klein AK, Dyck JA, Shimizu JA, Stitzel KA, Wilson FD, Cain GR. Effect of continuous, whole-body gamma irradiation upon canine lymphohematopoietic (CFU-GM, CFU-L) progenitors and a possible hematopoietic regulatory population. Radiat Res 101:332–350 (1985).
35. Dorshkind K, Johnson A, Harrison Y, Landreth KS. A colony assay system that detects B cell progenitors in fresh and cultured bone marrow. J Immunol Methods 123:93–101 (1989).
36. Johnson GR, Metcalf D. Pure and mixed erythroid colony formation *in vitro* stimulated by spleen conditioned medium with no detectable erythropoietin. Proc Natl Acad Sci USA 74:3879–3882 (1997).
37. Metcalf D, Johnson GR, Mandel TE. Colony formation in agar by multipotential hemopoietic cells. J Cell Physiol 98:401–420 (1979).
38. Williams N, Jackson H, Meyers P. Isolation of pluripotent hemopoietic stem cells and clonable precursor cells of erythrocytes, granulocytes, macrophages and megakaryocytes from mouse bone marrow. Exp Hematol 7:524–534 (1979).
39. Johnson GR. Colony formation in agar by adult bone marrow multipotential hemopoietic cells. J Cell Physiol 103:371–383 (1980).
40. Nishi N, Nakahata T, Koike K, Takagi M, Naganuma K, Akabane T. Induction of mixed erythroid-megakaryocyte colonies and bipotential blast cell colonies by recombinant human erythropoietin in serum-free culture. Blood 76:1330–1335 (1990).
41. Hara H, Ogawa M. Murine hemopoietic colonies in culture containing normoblasts, macrophages, and megakaryocytes. Am J Hematol 4:23–34 (1978).
42. Fauser AA, Messner HA. Identification of megakaryocytes, macrophages, and eosinophils in colonies of human bone marrow containing neutrophilic granulocytes and erythroblasts. Blood 53:1023–1027 (1979).
43. Bradley TR, Hodgson GS. Detection of primitive macrophage progenitor cells in mouse bone marrow. Blood 54:1446–1450 (1979).
44. Baines P, Bol S, Rosendaal M. Characterization of a developmentally early macrophage progenitor found in normal mouse marrow. Br J Haematol 48:147–153 (1981).
45. Baines P, Lajmanovich A, Hollard D. Enrichment of high proliferation potential colony forming cells from mouse marrow by selecting low-density cells expressing receptors for wheat germ agglutinin. Leuk Res 8:853–861 (1984).
46. Hodgson GS, Bradley TR, Radley JM. The organization of hemopoietic tissue as inferred from the effects of 5-fluorouracil. Exp Hematol 10:26–35 (1982).
47. Boswell HS, Wade PM Jr, Quesenberry PJ. Thy-1 antigen expression by murine high-proliferative capacity hematopoietic progenitor cells. I: Relation between sensitivity to depletion by Thy-1 antibody and stem cell generation potential. J Immunol 133:2940–2949 (1984).
48. Leary AG, Ogawa M. Blast cell colony assay for umbilical cord blood and adult bone marrow progenitors. Blood 69:953–956 (1987).
49. McNiece IK, Stewart FM, Deacon DM, Temeles DS, Zsebo KM, Clark SC, Quesenberry PJ. Detection of a human CFC with a high proliferative potential. Blood 74:609–612 (1989).
50. McNiece IK, Bertoncello I, Kriegler AB, Quesenberry PJ. Colony-forming cells with high proliferative potential (HPP-CFC). Int J Cell Cloning 8:146–160 (1990).
51. Ploemacher RE, van der Sluijs JP, Voerman JS, Brons NH. An *in vitro* limiting-dilution assay of long-term repopulating hematopoietic stem cells in the mouse. Blood 74:2755–2763 (1989).
52. Sutherland HJ, Lansdorp PM, Henkelman DH, Eaves AC, Eaves CJ. Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. Proc Natl Acad Sci USA 87:3584–3588 (1990).
53. Pettengell R, Luft T, Henschler R, Hows JM, Dexter TM, Ryder D, Testa NG. Direct comparison by limiting dilution analysis of long-term culture-initiating cells in human bone marrow, umbilical cord blood, and blood stem cells. Blood 84:3653–3659 (1994).
54. Breems DA, Blokland EA, Neben S, Ploemacher RE. Frequency analysis of human primitive haematopoietic stem cell subsets using a cobblestone area forming cell assay. Leukemia 8:1095–1104 (1994).
55. Eaves CJ, Sutherland HJ, Udomsakdi C, Lansdorp PM, Szilvassy SJ, Fraser CC, Humphries RK, Barnett MJ, Phillips GL, Eaves AC. The human hematopoietic stem cell *in vitro* and *in vivo*. Blood Cells 18:301–307 (1992).
56. Lemieux ME, Rebel VI, Lansdorp PM, Eaves CJ. Characterization and purification of a primitive hematopoietic cell type in adult mouse marrow capable of lymphomyeloid differentiation in long-term marrow switch cultures. Blood 86:1339–1347 (1995).

57. Ploemacher RE, Brons RH. Separation of CFU-S from primitive cells responsible for reconstitution of the bone marrow hemopoietic stem cell compartment following irradiation: evidence for a pre-CFU-S cell. Exp Hematol 17:263–266 (1989).
58. Murray L, DiGiusto D, Chen B, Chen S, Combs J, Conti A, Galy A, Negrin R, Tricot G, Tsukamoto A. Analysis of human hematopoietic stem cell populations. Blood Cells 20:364–369 (1994).
59. Orlic D, Fischer R, Nishikawa S, Nienhuis AW, Bodine DM. Purification and characterization of heterogeneous pluripotent hematopoietic stem cell populations expressing high levels of c-*kit* receptor. Blood 82:762–770 (1993).
60. Orlic D, Anderson S, Bodine DM. Biological properties of subpopulations of pluripotent hematopoietic stem cells enriched by elutriation and flow cytometry. Blood Cells 20:107–117 (1994).
61. Watt SM, Visser JW. Recent advances in the growth and isolation of primitive human haemopoietic progenitor cells. Cell Prolif 25:263–297 (1992).
62. Zijlmans JM, Visser JW, Kleiverda K, Kluin PM, Willemze R, Fibbe WE. Modification of rhodamine staining allows identification of hematopoietic stem cells with preferential short-term or long-term bone marrow-repopulating ability. Proc Natl Acad Sci USA 92:8901–8905 (1995).
63. Berardi AC, Wang A, Levine JD, Lopez P, Scadden DT. Functional isolation and characterization of human hematopoietic stem cells. Science 267:104–108 (1995).
64. Bungart B, Loeffler M, Goris H, Dontje B, Diehl V, Nijhof W. Differential effects of recombinant human colony stimulating factor (rh G-CSF) on stem cells in marrow, spleen and peripheral blood in mice. Br J Haematol 76:174–179 (1990).
65. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning *in vitro* and retransplantation *in vivo*. Transplantation 17:331–340 (1974).
66. Van Den Heuvel R, Schoeters G, Leppens H, Vanderborght O. Stromal cells in long-term cultures of liver, spleen, and bone marrow at different developmental ages have different capacities to maintain GM-CFC proliferation. Exp Hematol 19:115–121 (1991).
67. Aizawa S, Yaguchi M, Nakano M, Toyama K, Inokuchi S, Imai T, Yasuda M, Nabeshima R, Handa H. Hematopoietic supportive function of human bone marrow stromal cell lines established by a recombinant SV40-adenovirus vector. Exp Hematol 22:482–487 (1994).
68. Friedenstein AJ, Deriglasova UF, Kulagina NN, Panasuk AF, Rudakowa SF, Luria EA, Ruadkow IA. Precursors for fibroblasts in different populations of hematopoietic cells as detected by the *in vitro* colony assay method. Exp Hematol 2:83–92 (1974).
69. Dexter TM. Haemopoiesis in long-term bone marrow cultures. A review. Acta Haematol 62:299–305 (1979).
70. Gartner S, Kaplan HS. Long-term culture of human bone marrow cells. Proc Natl Acad Sci USA 77:4756–4759 (1980).
71. Johnson A, Dorshkind K. Stromal cells in myeloid and lymphoid long-term bone marrow cultures can support multiple hemopoietic lineages and modulate their production of hemopoietic growth factors. Blood 68:1348–1354 (1986).
72. Wang TY, Brennan JK, Wu JH. Multilineal hematopoiesis in a three-dimensional murine long-term bone marrow culture. Exp Hematol 23:26–32 (1995).
73. Whitlock CA, Witte ON. Long-term culture of B lymphocytes and their precursors from murine bone marrow. Proc Natl Acad Sci USA 79:3608–3612 (1982).
74. Nagasawa R, Kanagawa O, Tittle TV, Chiller JM. *In vivo* maturation of pre-B cells derived from long-term cultured bone marrow. J Immunol 135:965–972 (1985).
75. Whitlock CA, Witte ON. Long-term culture of murine bone marrow precursors of B lymphocytes. Methods Enzymol 150:275–286 (1987).
76. Dorshkind K. *In vitro* differentiation of B lymphocytes from primitive hemopoietic precursors present in long-term bone marrow cultures. J Immunol 136:422–429 (1986).
77. Collins LS, Dorshkind K. A stromal cell line from myeloid long-term bone marrow cultures can support myelopoiesis and B lymphopoiesis. J Immunol 138:1082–1087 (1987).
78. Greenberg PL, Schrier SL. Clinical utility of *in vitro* evaluation of granulopoiesis. Annu Rev Med 25:269–278 (1974).
79. Marsh JC. Chemical toxicity of the granulocyte. Environ Health Perspect 39:71–78 (1981).
80. Boorman GA, Luster MI, Dean JH, Campbell ML. Assessment of myelotoxicity caused by environmental chemicals. Environ Health Perspect 43:129–135 (1982).
81. Deldar A, Lewis H, Bloom J, Weiss L. Reproducible cloning assays for *in vitro* growth of canine hematopoietic progenitor cells and their potential applications in investigative hematotoxicity. Am J Vet Res 49:1393–1401 (1988).
82. Naughton BA, Sibanda B, Azar L, San Roman J. Differential effects of drugs upon hematopoiesis can be assessed in long-term bone marrow cultures established on nylon screens. Proc Soc Exp Biol Med 199:481–490 (1992).
83. Noble C, Sina JF. Usefulness of the *in vitro* bone marrow colony-forming assay in cellular toxicology. In Vitro Toxicol 6:187–195 (1993).
84. Parchment RE, Huang M, Erickson-Miller CL. Roles for *in vitro* myelotoxicity tests in preclinical drug development and clinical trial planning. Toxicol Pathol 21:241–250 (1993).
85. Deldar A, Stevens CE. Development and application of *in vitro* models of hematopoiesis to drug development. Toxicol Pathol 21:231–240 (1993).
86. Deldar A. Drug-induced blood disorders: review of pathogenetic mechanisms and utilisation of bone marrow cell culture technology as an investigative approach. Curr Top Vet Res 1:83–101 (1994).
87. Deldar A, Parchment RE. Preclinical risk assessment for hematotoxicity: animal models and *in vitro* systems. In: Comprehensive Toxicology (Sipes GI, McQueen CA, Gandolfi AJ, eds). Vol 4: Toxicology of the Hematopoietic System (Bloom JC, ed). New York:Pergamon, 1997;303–320.
88. Parchment RE, Murphy MJ Jr. Human hematopoietic stem cells: laboratory assessment and response to toxic injury. In: Comprehensive Toxicology (Sipes GI, McQueen CA, Gandolfi AJ, eds). Vol 4: Toxicology of the Hematopoietic System (Bloom JC, ed). New York:Pergamon, 1997;335–362.
89. Treleaven J, Barrett J. Drugs and the bone marrow. Br J Hosp Med 44:245–250 (1990).
90. Greenberger JS. Toxic effects on the hematopoietic microenvironment. Exp Hematol 19:1101–1109 (1991).
91. Young GA, Croaker G, Vincent PC, Forrest P, Morris TC. The CFU-C assay in patients with neutropenia and, in particular, drug associated neutropenia. Clin Lab Haematol 9:245–253 (1987).
92. Naughton BA, San Roman J, Sibanda B, Weintraub JP, Kamali V. Stereotypic culture systems for liver and bone marrow: evidence for the development of cuntional tissue *in vitro* and following implantation *in vivo*. Biotechnol Bioeng 43:810–825 (1994).
93. Bloom JC. Introduction to hematotoxicology. In: Comprehensive Toxicology (Sipes GI, McQueen CA, Gandolfi AJ, eds). Vol 4: Toxicology of the Hematopoietic System (Bloom JC, ed). New York:Pergamon, 1997;1–10.
94. Levitt LJ, Quesenberry PJ. The effect of lithium on murine hematopoiesis in a liquid culture system. N Engl J Med 302:713–719 (1980).
95. Chatelain C, Burstein SA, Harker LA. Lithium enhancement of megakaryocytopoiesis in culture: mediation via accessory marrow cells. Blood 62:172–176 (1983).
96. Boggs DR, Joyce RA. The hematopoietic effects of lithium. Semin Hematol 20:129–138 (1983).
97. Silberstein DS, Austen KF, Owen WF Jr. Hemopoietins for eosinophils. Glycoprotein hormones that regulate the development of inflammation in eosinophilia-associated disease. Hematol Oncol Clin North Am 3:511–533 (1989).
98. Owen WF Jr, Petersen J, Sheff DM, Folkerth RD, Anderson RJ, Corson JM, Sheffer AL, Austen KF. Hypodense eosinophils and interleukin 5 activity in the blood of patients with the

eosinophilia-myalgia syndrome. Proc Natl Acad Sci USA 87:8647–8651 (1990).
99. Yamaoka KA, Miyasaka N, Inuo G, Saito I, Kolb JP, Fujita K, Kashiwazaki S. 1,1′-Ethylidenebis(tryptophan) (Peak E) induces functional activation of human eosinophils and interleukin 5 production from T lymphocytes: association of eosinophilia-myalgia syndrome with a L-tryptophan contaminant. J Clin Immunol 14:50–60 (1994).
100. Gallamini A, Carbone A, Lista P, Cavallero G, Reato G, Fruttero A, Novero D, Asnaghi G, di Celle PF, Foa R. Intestinal T-cell lymphoma with massive tissue and blood eosinophilia mediated by IL-5. Leuk Lymphoma 17:155–161 (1995).
101. Macdonald D, Gordon AA, Kajitani H, Enokihara H, Barrett AJ. Interleukin-2 treatment-associated eosinophilia is mediated by interleukin-5 production. Br J Haematol 76:168–173 (1990).
102. Dale DC, Fauci AS, Guerry D IV, Wolff SM. Comparison of agents producing a neutrophilic leukocytosis in man: hydrocortisone, prednisone, endotoxin, and etiocholanolone. J Clin Invest 56:808–813 (1975).
103. Grillot-Courvalin C, Vinci G, Tsapis A, Dokhelar MC, Vainchenker W, Brouet JC. The syndrome of T8 hyperlymphocytosis: variation in phenotype and cytotoxic activities of granular cells and evaluation of their role in associated neutropenia. Blood 69:1204–1210 (1987).
104. van der Veen JP, Goldschmeding R, Miedema F, Smit JW, Melief CJ, von dem Borne AE. K-cell lymphocytosis/neutropenia syndrome: the neutropenia is not caused by autoimmunity. Br J Haematol 64:777–787 (1986).
105. Smith JG, Smith MA, James I, Blundell E, Maddison PJ. Inhibition of CFU-GM by prostaglandins in a case of chronic T-cell lymphocytosis and neutropenia. Br J Haematol 73:148–151 (1989).
106. Greenberg P, Bax I, Mara B, Schrier S. Alterations of granulopoiesis following chemotherapy. Blood 44:375–383 (1974).
107. Bruce WR, Meeker BE, Valeriote FA. Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered *in vivo*. J Natl Cancer Inst 37:233–245 (1966).
108. Valeriote F, van Putten L. Proliferation-dependent cytotoxicity of anticancer agents: a review. Cancer Res 35:2619–2630 (1975).
109. Gallini R, Hendry JH, Molineux G, Testa NG. The effect of low dose rate on recovery of hemopoietic and stromal progenitor cells in gamma-irradiated mouse bone marrow. Radiat Res 115:481–487 (1988).
110. Horikoshi A, Murphy MJ Jr. Comparative effects of chemotherapeutic drugs on human and murine hematopoietic progenitors *in vitro*. Chemotherapy 28:480–501 (1982).
111. Qi DY, Hendry JH, Testa NG. Interactions in recovery and in residual injury from sequential treatments of mouse haemopoietic and stromal marrow cell populations, using X-rays, cyclophosphamide and busulphan. Radiother Oncol 20:46–52 (1991).
112. Seed TM, Kaspar LV. Changing patterns of radiosensitivity of hematopoietic progenitors from chronically irradiated dogs prone either to aplastic anemia or to myeloproliferative disease. Leuk Res 14:299–307 (1990).
113. Pessina A, Neri MG, Muschiato A, Raimondi A. Inhibition of granulocytic-macrophagic precursor cells (CFU-C) by heat-labile enterotoxin (LT) produced by *Escherichia coli*. Biomed Pharmacother 37:447–452 (1983).
114. Parent-Massin D, Thouvenot D. *In vitro* study of pesticide hematotoxicity in human and rat progenitors. J Pharmacol Toxicol Methods 30:203–207 (1993).
115. Parent-Massin D, Fuselier R, Thouvenot D. *In vitro* toxicity of trichothecenes on human haematopoietic progenitors. Food Addit Contam 11:441–447 (1994).
116. Inoue T, Tsushita K, Itoh T, Ogura M, Hotta T, Saneyoshi M, Yoshida S, Saitoh H, Tomoda Y, Nagai Y. *In vitro* bone marrow toxicity of nucleoside analogs against human immunodeficiency virus. Antimicrob Agents Chemother 33:576–579 (1989).
117. Fink FM, Maurer-Dengg K, Fritsch G, Mann G, Zoubek A, Falk M, Gadner H. Recombinant human granulocyte-macrophage colony-stimulating factor in septic neutropenic pediatric cancer patients: detection of circulating hematopoietic precursor cells correlates with rapid granulocyte recovery. Med Pediatr Oncol 25:365–371 (1995).
118. Wittels B. Bone marrow biopsy changes following chemotherapy for acute leukemia. Am J Surg Pathol 4:35–142 (1980).
119. Schurig JE, Schlein A, Florczyk AP, Farwell AR, Bradner WT. Animal models for evaluating the myelosuppressive effects of cancer chemotherapeutic agents. Exp Hematol 13(Suppl 16):101–105 (1985).
120. Ventura GJ, Reading CL, Hester JP, Vadhan-Raj S. Circulating myeloid progenitor cell kinetics during hematologic recovery from chemotherapy and subsequent recombinant human granulocyte-macrophage colony-stimulating factor administration. Acta Haematol 84:175–181 (1990).
121. Ema H, Suda T, Sakamoto S, Tomonaga T, Tsunoda J, Muroi K, Komatsu N, Miwa A, Ohsaka A, Yoshida M et al. Effects of the *in vivo* administration of recombinant human granulocyte colony-stimulating factor following cytotoxic chemotherapy on granulocytic precursors in patients with malignant lymphoma. Jpn J Cancer Res 80:577–582 (1989).
122. Ponassi A, Morra L, Mela GS, Caristo G, Parodi GB, Sacchetti C. Correlation between blood granulocyte progenitor cells and polymorphonuclear leukocytes. A tentative pathophysiological subgrouping of neutropenic and neutrophilic patients. Biomed Pharmacother 37:293–297 (1983).
123. Mukai J, Shimizu E, Ogura T. Granulocyte-colony-stimulating factor enhances the circulating hematopoietic progenitors in lung cancer patients treated with cisplatin-containing regimens. Jpn J Cancer Res 83:746–753 (1992).
124. Neelis KJ, Dubbelman YD, Luo QL, Thomas GR, Eaton DL, Wagemaker G. Simultaneous administration of TPO and G-CSF after cytoreductive treatment of rhesus monkeys prevents thrombocytopenia, accelerates platelet and red cell reconstitution, alleviates neutropenia, and promotes the recovery of immature bone marrow cells. Exp Hematol 25:1084–1093 (1997).
125. Du DL, Volpe DA, Grieshaber CK, Murphy MJ Jr. Comparative toxicity of fostriecin, hepsulfam and pyrazine diazohydroxide to human and murine hematopoietic progenitor cells *in vitro*. Invest New Drugs 9:149–157 (1991).
126. Hendricks CB, Grochow LB, Rowinsky EK, Forastiere AA, McGuire WP, Ettinger DS, Sartorius S, Lubejko B, Donehower RC. Phase I and pharmacokinetic study of hepsulfam (NSC 329680). Cancer Res 51:5781–5785 (1991).
127. Volpe DA, Du DL, Zurlo MG, Mongelli N, Murphy MJ Jr. Comparative *in vitro* myelotoxicity of FCE 24517, a distamycin derivative, to human, canine and murine hematopoietic progenitor cells. Invest New Drugs 10:255–261 (1992).
128. Volpe DA, Du DL, Grieshaber CK, Murphy MJ Jr. *In vitro* characterization of the myelotoxicity of cyclopentenyl cytosine. Cancer Chemother Pharmacol 34:103–108 (1994).
129. Parchment RE, Volpe DA, LoRusso PM, Erickson-Miller CL, Murphy MJ Jr, Grieshaber CK. *In vivo-in vitro* correlation of myelotoxicity of 9-methoxypyrazoloacridine (NSC-366140, PD115934) to myeloid and erythroid hematopoietic progenitors from human, murine, and canine marrow. J Natl Cancer Inst 86:273–280 (1994).
130. Volpe DA, Cole K, Sandeen MA, Kohn EC. *In vitro* and *in vivo* myelotoxicity of CAI to human and murine hematopoietic progenitor cells. Am J Hematol 50:277–282 (1995).
131. Du DL, Volpe DA, Grieshaber CK, Murphy MJ Jr. Effects of L-phenylalanine mustard and L-buthionine sulfoximine on murine and human hematopoietic progenitor cells *in vitro*. Cancer Res 50:4038–4043 (1990).
132. Smith AC, Liao JT, Page JG, Wientjes MG, Grieshaber CK. Pharmacokinetics of buthionine sulfoximine (NSC 326231) and its effect on melphalan-induced toxicity in mice. Cancer Res 49:5385–5391 (1989).

133. Pressacco J, Erlichman C. Combination studies with 3′-azido-3′-deoxythymidine (AZT) plus ICI D1694. Cytotoxic and biochemical effects. Biochem Pharmacol 46:1989–1997 (1993).
134. Pressacco J, Hedley DW, Erlichman C. ICI D1694 and idoxuridine: a synergistic antitumor combination. Cancer Res 54:3772–3778 (1994).
135. Kalechman Y, Sotnik-Barkai I, Albeck M, Sredni B. Protection of bone marrow stromal cells from the toxic effects of cyclophosphamide *in vivo* and of ASTA-Z 7557 and etoposide *in vitro* by ammonium trichloro(dioxyethylene-*O*-*O*′)tellurate (AS101). Cancer Res 53:1838–1844 (1993).
136. Kalechman Y, Rushkin G, Nerubay J, Albeck M, Sredni B. Effect of the immunomodulator AS101 on chemotherapy-induced multilineage myelosuppression, thrombocytopenia, and anemia in mice. Exp Hematol 23:1358–1366 (1995).
137. Walker RE, Parker RI, Kovacs JA, Masur H, Lane HC, Carleton S, Kirk LE, Gralnick HR, Fauci AS. Anemia and erythropoiesis in patients with the acquired immunodeficiency syndrome (AIDS) and Kaposi sarcoma treated with zidovudine. Ann Intern Med 108:372–376 (1988).
138. Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, Leedom JM, Groopman JE, Mildvan D, Hirsch MS et al. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N Engl J Med 317:192–197 (1987).
139. Dainiak N, Worthington M, Riordan MA, Kreczko S, Goldman L. 3′-Azido-3′-deoxythymidine (AZT) inhibits proliferation *in vitro* of human haematopoietic progenitor cells. Br J Haematol 69:299–304 (1988).
140. Morse GD, Olson J, Portmore A, Taylor C, Plank C, Reichman RC. Pharmacokinetics of orally administered zidovudine among patients with hemophilia and asymptomatic human immunodeficiency virus (HIV) infection. Antiviral Res 11:57–65 (1989).
141. Bhalla K, Birkhofer M, Grant S, Graham G. The effect of recombinant human granulocyte-macrophage colony-stimulating factor (rGM-CSF) on 3′-azido-3′-deoxythymidine (AZT)-mediated biochemical and cytotoxic effects on normal human myeloid progenitor cells. Exp Hematol 17:17–20 (1989).
142. Balis FM, Pizzo PA, Eddy J, Wilfert C, McKinney R, Scott G, Murphy RF, Jarosinski PF, Falloon J, Poplack DG. Pharmacokinetics of zidovudine administered intravenously and orally in children with human immunodeficiency virus infection. J Pediatr 114:880–884 (1989).
143. Collins JM, Unadkat JD. Clinical pharmacokinetics of zidovudine. An overview of current data. Clin Pharmacokinet 17:1–9 (1989).
144. Ganser A, Greher J, Volkers B, Staszewski S, Hoelzer D. Inhibitory effect of azidothymidine, 2′-3′-dideoxyadenosine, and 2′-3′-dideoxycytidine on *in vitro* growth of hematopoietic progenitor cells from normal persons and from patients with AIDS. Exp Hematol 17:321–325 (1989).
145. Du DL, Volpe DA, Grieshaber CK, Murphy MJ Jr. *In vitro* myelotoxicity of 2′,3′-dideoxynucleosides on human hematopoietic progenitor cells. Exp Hematol 18:832–836 (1990).
146. Irvine AE, Morris TC, Kelly GJ, McCracken N. Ticarcillin-induced neutropenia corroborated by *in vitro* CFU-C toxicity. Acta Haematol 70:364–368 (1983).
147. Volpe DA, Du DL, Verhoef V, Murphy MJ Jr. Myelotoxicity of rifabutin and 3′-azido-3′-deoxythymidine, alone and in combination, to human hematopoietic progenitor cells *in vitro*. Pathobiology 61:77–82 (1993).
148. Haworth C, Morris-Jones PH, Testa NG. Long-term bone-marrow damage in children treated for ALL: evidence from *in vitro* colony assays (GM-CFC and CFUF). Br J Cancer 46:918–923 (1982).
149. Jacobsen SE, Keller JR, Ruscetti FW, Kondaiah P, Roberts AB, Falk L.A. Bidirectional effects of transforming growth factor beta (TGF-beta) on colony-stimulating factor-induced human myelopoiesis *in vitro*: differential effects of distinct TGF-beta isoforms. Blood 78:2239–2247 (1991).
150. Piacibello W, Ferrero D, Sanavio F, Badoni R, Stacchini A, Severino A, Aglietta M. Responsiveness of highly enriched CFU-GM subpopulations from bone marrow, peripheral blood, and cord blood to hemopoietic growth inhibitors. Exp Hematol 19:1084–1089 (1991).
151. Ruscetti FW, Dubois C, Falk LA, Jacobsen SE, Sing G, Longo DL, Wiltrout RH, Keller JR. *In vivo* and *in vitro* effects of TGF-beta 1 on normal and neoplastic haemopoiesis. Ciba Found Symp 157:212–227 (1991).
152. Caux C, Moreau I, Saeland S, Banchereau J. Interferon-gamma enhances factor-dependent myeloid proliferation of human $CD34^+$ hematopoietic progenitor cells. Blood 79:2628–2635 (1992).
153. Broxmeyer HE, Lu L, Platzer E, Juliano L, Rubin BY. Comparative analysis of the influences of human gamma, alpha and beta interferons on human multipotential (CFU-GEMM), erythroid (BFU-E) and granulocyte-macrophage (CFU-GM) progenitor cells. J Immunol 131:1300–1305 (1983).
154. Cashman JD, Eaves AC, Raines EW, Ross R, Eaves CJ. Mechanisms that regulate the cell cycle status of very primitive hematopoietic cells in long-term human marrow cultures. I: Stimulatory role of a variety of mesenchymal cell activators and inhibitory role of TGF-beta. Blood 75:96–101 (1990).
155. Strife A, Lambek C, Perez A, Darzynkiewicz Z, Skierski J, Gulati S, Haley JD, ten Dijke P, Iwata KK, Clarkson BD. The effects of transforming growth factor beta 3 on the growth of highly enriched hematopoietic progenitor cells derived from normal human bone marrow and peripheral blood. Cancer Res 51:4828–4836 (1991).
156. Bursuker I, Neddermann KM, Petty BA, Schacter B, Spitalny GL, Tepper MA, Pasternak RD. *In vivo* regulation of hemopoiesis by transforming growth factor beta 1: stimulation of GM-CSF- and M-CSF-dependent murine bone marrow precursors. Exp Hematol 20:431–435 (1992).
157. Hargis JB, La Russa VF, Redmond J, Kessler SW, Wright DG. Agranulocytosis associated with Mexican aspirin (dipyrone): evidence for an autoimmune mechanism affecting multipotential hematopoietic progenitors. Am J Hematol 31:213–215 (1989).
158. Hauser SP, Udupa KB, Lipschitz DA. Effects of ceftazidime, a betalactam antibiotic, on murine haemopoiesis *in vitro*. Br J Haematol 86:733–739 (1994).
159. Stojanovic N, Ruvidic R, Jovcic G, Mijovic A. Drug-induced agranulocytosis: bone marrow granulocytic progenitor cells. Biomed Pharmacother 44:181–184 (1990).
160. Parmentier C, Tchernia G, Subtil E, Diakhate L, Morardet N. *In vitro* medullary granulocytic progenitor (CFUc) cultures from 6 cases of granulocytopenias. Scand J Haematol 21:19–23 (1978).
161. Pisciotta AV, Konings SA, Ciesemier LL, Cronkite CE, Lieberman JA. Cytotoxic activity in serum of patients with clozapine-induced agranulocytosis. J Lab Clin Med 119:254–266 (1992).
162. Pisciotta AV, Konings SA, Ciesemier LL, Cronkite CE, Lieberman JA. On the possible mechanisms and predictability of clozapine-induced agranulocytosis. Drug Saf 7(Suppl 1):33–44 (1992).
163. Levitt LJ. Chlorpropamide-induced pure white cell aplasia. Blood 69:394–400 (1987).
164. Parent-Massin DM, Sensebe L, Leglise MC, Guern G, Berthou C, Riche C, Abgrall JF. Relevance of *in vitro* studies of drug-induced agranulocytosis. Report of 14 cases. Drug Saf 9:463–469 (1993).
165. Gerson SL, Arce C, Meltzer HY. *N*-desmethylclozapine: a clozapine metabolite that.suppresses haemopoiesis. Br J Haematol 86:555–561 (1994).
166. Aymard JP, Rouveix B, Ferry R, Janot C, May T, Legras B, Streiff F. Amodiaquine-induced agranulocytosis: report of a case with *in vitro* studies of granulocyte-macrophage progenitor cells. Acta Haematol 82:40–42 (1989).
167. Aymard JP, Wioland C, Ferry R, Netter P, Streiff F. The *in vitro* effect of amodiaquine on bone marrow granulocyte-macrophage progenitor cells from normal subjects. Fundam Clin Pharmacol 6:1–4 (1992).

168. Ono K, Kurohara K, Yoshihara M, Shimamoto Y, Yamaguchi M. Agranulocytosis caused by ticlopidine and its mechanism. Am J Hematol 37:239–242 (1991).
169. Humphrey CA, French A, Morris TC. Prospective *in vitro* testing for drug-induced neutropenia in a patient requiring antimalarial prophylaxis: confirmation of findings on exposure of patient to drug. Clin Lab Haematol 12:31–36 (1990).
170. Schreml W, Lohrmann HP. No effects of levamisole on cytotoxic drug-induced changes of human granulopoiesis. Blut 38:331–336 (1979).
171. Sperner-Unterweger B, Gaggl S, Fleischhacker WW, Barnas C, Herold M, Geissler D. Effects of clozapine on hematopoiesis and the cytokine system. Biol Psychiatry 34:536–543 (1993).
172. Fibbe WE, Claas FH, Van der Star-Dijkstra W, Schaafsma MR, Meyboom RH, Falkenburg JH. Agranulocytosis induced by propylthiouracil: evidence of a drug dependent antibody reacting with granulocytes, monocytes and haematopoietic progenitor cells. Br J Haematol 64:363–373 (1986).
173. Bloom JC, Thiem PA, Sellers TS, Deldar A, Lewis HB. Cephalosporin-induced immune cytopenia in the dog: demonstration of erythrocyte-, neutrophil-, and platelet-associated IgG following treatment with cefazedone. Am J Hematol 28:71–78 (1988).
174. Kobashi H, Adachi T, Tsubota T, Asano K, Fukai M, Namba J, Izumi K, Hoshijima T, Miura H, Sezaki T. The role of drugs and lymphocytes in granulocyte-macrophage colony formation in patients with drug induced agranulocytosis [in Japanese]. Rinsho Ketsueki 30:282–288 (1989).
175. Matsuzaki M, Tokioka T, Suga K, Sueoka E, Ono K, Sano M, Shimamoto Y, Yamaguchi M. Phenytoin induced agranulocytosis: direct and T cell-mediated mechanisms [in Japanese]. Rinsho Ketsueki 31:474–478 (1990).
176. Douer D, Eisenstein Z. Methimazole-induced agranulocytosis: growth inhibition of myeloid progenitor cells by the patient's serum. Eur J Haematol 40:91–94 (1988).
177. Pessina A, Neri MG, Muschiato A, Mineo E, Cocuzza G. Effect of fluoroquinolones on the in-vitro proliferation of myeloid precursor cells. J Antimicrob Chemother 24:203–208 (1989).
178. Deldar A, House RV, Wierda D. Bone marrow colony-forming assays. Methods Immunotoxicol 1:227–250 (1995).
179. Volpe DA, Du DL, Pohl KP, Campbell JP, Murphy MJ Jr. Utility of human bone marrow obtained incidental to orthopedic surgery for hematopoietic clonal assays. Pathobiology 59:53–56 (1991).
180. Du DL, Volpe DA, Murphy MJ Jr. Microcapillary clonogenic assays for human marrow hematopoietic progenitor cells. Int J Cell Cloning 7:303–313 (1989).
181. Meijne EI, Ploemacher RE, Vos O, Huiskamp R. The effects of graded doses of 1 MeV fission neutrons or X rays on the murine hematopoietic stroma. Radiat Res 131:302–308 (1992).
182. Xu CX, Hendry JH, Testa NG. The response of stromal progenitor cells in mouse marrow to graded repeated doses of X rays or neutrons. Radiat Res 96:82–89 (1983).
183. Klein AK, Rosenblatt LS, Stitzel KA, Greenberg B, Woo L. *In vitro* radiation response studies on bone marrow fibroblasts (CFU-F) obtained from normal and chronically irradiated dogs. Leuk Res 8:473–481 (1984).
184. Hendry JH, Wang SB, Testa NG. Greater sparing of stromal progenitor cells than of haemopoietic stem cells in gamma-irradiated mouse marrow using low dose-rates. Biomed Pharmacother 38:356–358 (1984).
185. Zhu H, Li Y, Trush MA. Characterization of benzo[*a*]pyrene quinone-induced toxicity to primary cultured bone marrow stromal cells from DBA/2 mice: potential role of mitochondrial dysfunction. Toxicol Appl Pharmacol 130:108–120 (1995).
186. Abraham NG, Bucher D, Niranjan U, Brown AC, Lutton JD, Distenfeld A, Ahmed T, Levere RD. Microenvironmental toxicity of azidothymidine: partial sparing with hemin. Blood 74:139–144 (1989).
187. Ben-Ishay Z, Prindull G, Sharon S, Borenstein A. Effects of chemotherapy on bone marrow stroma in mice with acute myelogenous leukemia. Correlation with CFU-C and CFU-D. Leuk Res 9:1059–1067 (1985).
188. Gidali J, Istvan E, Feher I. Long-term perturbation of hemopoiesis after moderate damage to stem cells. Exp Hematol 13:647–651 (1985).
189. Domenech J, Gihana E, Dayan A, Truglio D, Linassier C, Desbois I, Lamagnere JP, Colombat P, Binet C. Haemopoiesis of transplanted patients with autologous marrows assessed by long-term marrow culture. Br J Haematol 88:488–496 (1994).
190. Betticher DC, Huxol H, Muller R, Speck B, Nissen C. Colony growth in cultures from bone marrow and peripheral blood after curative treatment for leukemia and severe aplastic anemia. Exp Hematol 21:1517–1521 (1993).
191. Chang J, Geary CG, Testa NG. Long-term bone marrow damage after chemotherapy for acute myeloid leukemia does not improve with time. Br J Haematol 75:68–72 (1990).
192. Radford JA, Testa NG, Crowther D. The long-term effects of MVPP chemotherapy for Hodgkin's disease on bone marrow function. Br J Cancer 62:127–132 (1990).
193. Grande T, Gaitan S, Tejero C, Bueren JA. Residual haematopoietic damage in adult and 8-day-old mice exposed to 7 Gy of X-rays. Int J Radiat Biol 63:59–67 (1993).
194. Grande T, Bueren JA. Involvement of the bone marrow stroma in the residual hematopoietic damage induced by irradiation of adult and young mice. Exp Hematol 22:1283–1287 (1994).
195. Grande T, Bueren JA. Analysis of hematopoiesis in mice irradiated with 500 mGy of X rays at different stages of development. Radiat Res 143:327–333 (1995).
196. Burke TG, Mi Z. Preferential binding of the carboxylate form of camptothecin by human serum albumin. Anal Biochem 212:285–287 (1993).
197. Burke TG, Mi Z. The structural basis of camptothecin interactions with human serum albumin: impact on drug stability. J Med Chem 37:40–46 (1994).
198. Mi Z, Burke TG. Differential interactions of camptothecin lactone and carboxylate forms with human blood components. Biochemistry 33:10325–10336 (1994).
199. Mi Z, Burke TG. Marked interspecies variations concerning the interactions of camptothecin with serum albumins: a frequency-domain fluorescence spectroscopic study. Biochemistry 33:12540–12545 (1994).
200. Burke TG, Munshi CB, Mi Z, Jiang Y. The important role of albumin in determining the relative human blood stabilities of the camptothecin anticancer drugs [letter]. J Pharm Sci 84:518–519 (1995).
201. Song D, Hsu LF, Au JL. Binding of taxol to plastic and glass containers and protein under *in vitro* conditions. J Pharm Sci 85:29–31 (1996).
202. Lopez RL, Peters GJ, van Loenen AC, Pizao PE, van Rijswijk REN, Wagstaff J, Pinedo HM. The effect of schedule, protein binding and growth factors on the activity of suramin. Int J Cancer 51:921–926 (1992).
203. Miller VA, Rigas JR, Tong WP, Reid JR, Pisters KMW, Grant SC, Heelan RT, Kris MG. Phase II trial of chloroquinoxaline sulfonamide (CQS) in patients with stage III and IV non-small-cell lung cancer. Cancer Chemother Pharmacol 40:415–418 (1997).
204. Myers SR, Flesher JW. Metabolism of the carcinogen 3-methylcholanthrene in human bone marrow preparations. Drug Metab Dispos 18:664–669 (1990).
205. Twerdok LE, Trush MA. Differences in quinone reductase activity in primary bone marrow stromal cells derived from C57BL/6 and DBA/2 mice. Res Commun Chem Pathol Pharmacol 67:375–386 (1990).
206. Thomas DJ, Sadler A, Subrahmanyam VV, Siegel D, Reasor MJ, Wierda D, Ross D. Bone marrow stromal cell bioactivation and detoxification of the benzene metabolite hydroquinone: comparison of macrophages and fibroblastoid cells. Mol Pharmacol 37:255–262 (1990).
207. Twerdok LE, Trush MA. Studies on biochemical determinants of quinone-induced toxicity in primary murine bone marrow stromal cells. Adv Exp Med Biol 283:843–846 (1991).

208. Twerdok LE, Mosebrook DR, Trush MA. Comparison of oxidant-generation and BP-diol activation by bone marrow cells from C57Bl/6 and DBA/2 mice: implications for risk of bone marrow toxicity induced by polycyclic hydrocarbons. Toxicol Appl Pharmacol 112:266–272 (1992).
209. Twerdok LE, Rembish SJ, Trush MA. Induction of quinone reductase and glutathione in bone marrow cells by 1,2-dithiole-3-thione: effect on hydroquinone-induced cytotoxicity. Toxicol Appl Pharmacol 112:273–281 (1992).
210. Ganousis LG, Goon D, Zyglewska T, Wu KK, Ross D. Cell-specific metabolism in mouse bone marrow stroma: studies of activation and detoxification of benzene metabolites. Mol Pharmacol 42:1118–1125 (1992).
211. Gianni L, Vigano L, Surbone A, Ballinari D, Casali P, Tarella C, Collins JM, Bonadonna G. Pharmacology and clinical toxicity of 4′-iodo-4′-deoxydoxorubicin: an example of successful application of pharmacokinetics to dose escalation in phase I trials. J Natl Cancer Inst 82:469–477 (1990).
212. Deldar A, Stevens CE, Beineke PJ. Comparative distribution of marrow CFU-e and CFU-gm progenitors in different anatomic sites in the dog. Int J Cell Cloning 8:196–208 (1990).
213. Deldar A, Stevens CE, Rodocker KB. Canine BFU-e progenitors: adaptation of a reproducible assay and anatomical distribution. Int J Cell Cloning 9:579–593 (1991).
214. Demirer T, Rowley S, Buckner CD, Appelbaum FR, Lilleby K, Storb R, Schiffman K, Bensinger WI. Peripheral-blood stem-cell collections after paclitaxel, cyclophosphamide, and recombinant human granulocyte colony-stimulating factor in patients with breast and ovarian cancer. J Clin Oncol 13:1714–1719 (1995).
215. Bender JG, Lum L, Unverzagt KL, Lee W, Van Epps D, George S, Coon J, Ghalie R, McLeod B, Kaizer H et al. Correlation of colony-forming cells, long-term culture initiating cells and $CD34^+$ cells in apheresis products from patients mobilized for peripheral blood progenitors with different regimens. Bone Marrow Transplant 13:479–485 (1994).
216. Urashima M, Uchiyama H, Hoshi Y, Deguchi Y, Kamijou M, Katou Y, Fujisawa K, Akatsuka J, Maekawa K. Prediction of engraftment after peripheral blood stem cell transplantation by CD34-positive cells in grafts. Acta Paediatr Jpn 35:325–331 (1993).
217. Holberg-Petersen M, Rollag H, Beck S, Øverli I, Tjønnfjord G, Abrahamsen TG, Degré M, Hestdal K. Direct growth suppression of myeloid bone marrow progenitor cells but not cord blood progenitors by human cytomegalovirus *in vitro*. Blood 88:2510–2516 (1996).
218. Leglise MC, de Tailly PD, Vignot JL, LeBot MA, LeRoux AM, Riche C. A cellular model for drug interactions on hematopoiesis: the use of human umbilical cord blood progenitors as a model for the study of drug-related myelosuppression of normal hematopoiesis. Cell Biol Toxicol 12:39–53 (1996).
219. Erickson-Miller CL, May RD, Tomaszewski J, Osborn B, Murphy MJ, Page JG, Parchment RE. Differential toxicity of camptothecin, topotecan and 9-aminocamptothecin to human, canine, and murine myeloid progenitors (CFU-GM) *in vitro*. Cancer Chemother Pharmacol 39:467–472 (1997).